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Original Article

The fear of unseen predators: ground squirrel tail flagging in the absence of snakes signals vigilance

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Rather than fleeing from predators, many animals exhibit conspicuous displays that are thought to deter attacks by advertising quality (physical capabilities), or awareness of the predator. However, prey commonly exhibit the same antipredator display when predators are absent. Signaling in this context could either dishonestly indicate predator detection, or honestly indicate vigilance, but no study has tested these hypotheses experimentally. We tested the vigilance advertisement function of tail flagging, a signal given by California ground squirrels (*Otospermophilus beecheyi*) to rattlesnakes (*Crotalus oreganus*), by simulating snake strikes and quantifying strike avoidance behaviors in signaling and non-signaling squirrels. We removed the effect of snake presence on signaling by simulating strikes 1) in the presence of a rattlesnake, 2) with no snake present, or 3) in an area where squirrels had recently encountered a snake that was no longer present. Squirrels tail flagged most when interacting with a snake. Squirrels also signaled in areas of recent snake encounters, and in this context, they responded faster to simulated strikes and were more likely to leap vertically or horizontally away from the attack. Tail flagging rate was also related to squirrels' body displacement time and flee modality. Squirrels rarely signaled or exhibited evasive leaping with no snake present. These results indicate that displays in the absence of predators can honestly signal vigilance, or the readiness to avoid an attack, even if predators are undetected. Such antipredator displays may be widespread as many species could benefit from advertising alertness when uncertain of the location of predators.

Key words: animal communication, *Crotalus oreganus*, honesty, *Otospermophilus beecheyi*, predator–prey interactions, pursuit-deterrent signal.

INTRODUCTION

Many animals of diverse taxa emit repetitive visual or vocal signals when threatened by predators. Several studies have demonstrated that by emitting such signals, the prey communicates information about its state both to the predator and to other potential prey in the vicinity, and the predator uses this information to update its hunting decision (Hasson 1991; Dall et al. 2005). If these signals are part of a communication system, they should indicate a decreased likelihood of successful attack for the predator (Maynard Smith and Harper 1995). Some studies have demonstrated that predators abandon pursuit of signalers after receiving their displays (Cresswell 1994; Zuberbuhler et al. 1999; Barbour and Clark 2012). Thus, these predator-directed signals are often called pursuit-deterrent signals (Woodland et al. 1980; Hasson et al. 1989; Hasson 1991).

Animals may communicate several aspects of their current state to predators. The most common hypotheses suggest that prey advertise either their discovery of the predator (perception advertisement), or a quality of body condition that indicates escape ability (quality advertisement) (Caro 1986; Hasson 1991; Cresswell 1994; Caro 2005; Bradbury and Vehrencamp 2011). However, many predator-directed signals are given in the absence of an obvious predator elicitor (e.g., Murphy 2006; Randler 2006; Murphy 2007; Pereyra and Morton 2010; Avellis 2011). This implies either that prey engage in dishonest or mistaken perception advertisement (Searcy and Nowicki 2005; Murphy 2007), or prey are advertising their vigilance (Randler 2006; Carder and Ritchison 2009).

Vigilance increases an animal's likelihood of detecting a predatory threat (Elgar 1989; Arenz and Leger 2000; Unck et al. 2009). Many studies equate vigilance with head-up scanning behavior, but animals have been shown to still maintain vigilance while performing other activities (e.g., foraging; Lima and Bednekoff 1999). Thus, we define vigilance as an animal's readiness for a predatory

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threat, measured by the ability to avoid a simulated attack. When uncertain about the presence of predators, prey can still benefit from advertising predator-directed vigilance if such signals deter hidden predators from attacking. Honesty of these signals may be maintained through increased time and energy invested in signaling or increased conspicuousness to other predators via the handicap principle (Zahavi 1977; Bergstrom and Lachmann 2001). Hence, honest signalers of vigilance should prove harder to catch for predators than animals that do not signal (Bergstrom and Lachmann 2001; Searcy and Nowicki 2005). Although these behaviors are widespread, no studies have experimentally tested the function of antipredator displays emitted in the absence of predators in any system.

California ground squirrels (*Otospermophilus beecheyi*) respond to snake predators (rattlesnakes, *Crotalus oreganus*, and gopher snakes, *Pituophis catenifer*) by emitting conspicuous tail flags, characterized by vertical side-to-side movements of the tail (Owings and Coss 1977; Hennessy et al. 1981; Swaisgood et al. 1999). In the presence of snakes, squirrels exhibit prolonged bouts of inspection and tail flagging directed at the snake. However, as in other prey–predator signaling systems, squirrels also tail flag frequently when there is no snake present (Hersek and Owings 1993). Given that most squirrel tail flagging occurs in short bouts when no snake is actually present, we remain uncertain as to what information the signal affords snakes, and whether the signal is an honest or deceptive advertisement of the perception of a snake. Natural observations have shown that after receiving displays from adult squirrels, free-ranging rattlesnakes usually relocate ambush sites (Barbour and Clark 2012), a response predicted by a perception advertisement function of the signal (i.e., the snake leaves because its ambush site has been discovered). This same study found that when snakes attempted to strike squirrels, tail-flagging squirrels were more likely to evade these strikes than non-tail-flagging squirrels (Barbour and Clark 2012), an observation consistent with the hypothesis that tail flagging indicates a higher degree of vigilance toward predators attacking from ambush. However, this study could not decouple the effects of snake detection from vigilance toward undetected snakes because all focal squirrels were in close proximity to hunting rattlesnakes. Hence, the function of this display in the absence of snakes is still unclear.

Squirrels almost always tail flag when they detect a snake (Owings and Coss 1977; Coss and Biardi 1997; Swaisgood et al. 2003; Owings and Coss 2008; Barbour and Clark 2012), and tail flagging episodes after snake detection may last for prolonged periods (greater than 10 min) with squirrels making several advances toward the snake (Owings and Coss 2008). Squirrels tail flagging in the absence of snakes usually direct signals toward microhabitats where snakes are often encountered (e.g., tall grass, burrows) or perhaps towards areas where the odor of a recently present snake is located (although this has not been tested experimentally). Tail flagging in the absence of snakes is qualitatively more subdued compared with displays given to snakes (Supplementary Video S1). Squirrels typically produce 1–2 tail flagging bouts before entering clumps of tall vegetation, whereas they may perform more than 30 bouts on detection of a snake (Hennessy et al. 1981). These differences in signal structure could relate to differences in signal function (snake detection vs. vigilance toward undetected snakes). In other animals, the functions of similar predator-directed signals change with context. For instance, black-tailed deer (*Odocoileus hemionus columbianus*) exhibit tail flagging over tail flicking when in a greater state of agitation and excitement (Stankowich 2008). Additionally,

tail undulations by broad-headed skinks (*Eumeces laticeps*) not only misdirect attacks of detected predators, but also may deflect attacks of undetected predators (Cooper 1998). Similarly, the functional significance of tail flagging may depend on squirrels' perception of snakes, but this needs experimental verification.

The goal of this study was to evaluate the function and honesty of a predator-directed signal through manipulative field experiments on free-ranging prey. We examined tail flagging occurrence and tail flagging rate by ground squirrels that 1) encountered a rattlesnake, 2) did not encounter a rattlesnake, or 3) had recently encountered a rattlesnake. Our methods allowed us to decouple the effects of snake perception from squirrel vigilance in the absence of snakes. We tested the vigilance advertisement function of tail flagging by simulating rattlesnake strikes toward squirrels under our 3 treatments. We hypothesized that, if tail flagging is an honest indicator of vigilance, signaling squirrels would evade simulated strikes more effectively than non-signalers.

MATERIALS AND METHODS

Study site

We performed this research at the Blue Oak Ranch Reserve (BORR), Santa Clara County, CA. BORR is at an elevation of approximately 800 m, and its habitat is characterized by steep to moderate hills covered by a mixed oak woodland landscape. We collected data from May to July in 2012 and 2013. All methods were approved by the San Diego State University Institutional Animal Care and Use Committee (APF 13-08-015C) and meet the ABS/ASAB guidelines for ethical treatment of animals.

Squirrel trapping and marking

We trapped adult squirrels continuously throughout the summers using Tomahawk traps baited with black oil sunflower seed. Once captured, squirrels were sedated with 40 mg/kg of Ketamine through injection into the hind leg muscle. We marked all squirrels with Nyanzol pelage dye for short-term visual identification, and metal ear tags for long-term identification. After processing, we kept squirrels captive until they regained normal movement, and then released them back at the point of capture within the same day of capture.

Rattlesnake collection and housing

Rattlesnakes used in our experimental trials were all wild caught, but differed in their time spent in captivity. Three snakes were long-term captives of the Owings Lab at the University of California, Davis (recently transferred to San Diego State University), whereas all others were found before and during the study at BORR by manual searching. We used a total of 11 snakes in this study, all of which were adult males. We kept snakes at the BORR field station throughout the duration of the study. They were housed in glass terraria with either paper towel or newspaper substratum, and provided with a hidebox and water ad libitum. Snakes were not fed during the 8 weeks of the study because food items in their stomachs would interfere with the tethering process (see below for details). It is normal for rattlesnakes, which are low-energy specialists, to fast for several weeks to months (McCue 2007). We attempted to only use large (>500 g) adult snakes for tethering, but because we could not locate enough large adults we used smaller adults for some trials. Long-term snake captives remained in captivity at the end of the study, whereas recently caught snakes from BORR were released at their place of capture.

Behavioral trials

We identified as many home burrows of marked squirrels as we could through natural observations. We habituated individual squirrels with identified home burrows to a specific location by repeatedly placing sunflower seed within a metal ring at that location (henceforth called the bait station). At each bait station, we examined the use of tail flagging and squirrel responses to simulated rattlesnake strikes under 1 of 3 treatments: 1) No Snake, 2) Snake Present, and 3) Recent Snake. For the No Snake treatment, squirrels were tested at their bait station with no snake present. For the Snake Present treatment, squirrels were tested at their bait station with a tethered rattlesnake present (see below for tethering details). For the Recent Snake treatment, squirrels were initially presented with a tethered rattlesnake at the bait station, and then we tested their responses on their next visit to the bait station after the snake was removed (less than 1 h after the snake encounter).

We tethered snakes for these experiments using a modified version of the technique described by Randall and Stevens (1987). We secured strings using either duct tape (2012) or athletic tape (2013) at 3 points on the body of the snake. These strings were then secured to tent stakes in the ground next to the bait station. Because snakes can overheat when exposed to direct sunlight, we provided them with shade (a 60 cm by 60 cm square of tarp tied to a PVC pipe frame) when bait stations were not covered by natural shade.

Snake strikes were simulated using a spring-loaded device that uncoils at approximately the same velocity as a rattlesnake strike. Studies on rattlesnake (*Crotalus atrox*, *Crotalus horridus*, *Crotalus viridis*) strike kinematics have reported predatory strike velocities at mean values ranging from 1.2 to 4.5 m/s (Cundall and Greene 2000; Cundall and Beaupre 2001; LaDuc 2002). Our device used a spring that uncoiled at 3.1 m/s. To create the strike-simulating device, we used a 90 cm long, 5 cm diameter spring topped with a 5.5 cm diameter cork. The spring was compressed into a 45 cm long, 5.7 cm diameter PVC pipe. For each trial, the device was secured into place with tent stakes 30 cm from the bait station. The cork-topped spring was held taut within the pipe using nylon monofilament fishing line secured next to an observer hidden behind an observation blind approximately 15 m from the focal squirrel's bait station. When the focal squirrel came into position in front of the device, the observer cut the line, thereby causing the spring to rapidly uncoil in the direction of the squirrel. We recorded the squirrels' responses to these

simulated strikes using a high frame rate video camera (120 frames per second, Sony model DCR SR-200). We also recorded focal squirrels as they approached their bait station using standard video cameras (30 frames per second, Sony Handycam model DCR-SR 85) in order to capture their tail flagging behaviors before the simulated attack. Prior to each trial, all the physical materials associated with the experiment (strike-simulating device, observation blind, snake shade) were put in place and focal squirrels were allowed to visit the bait station with these objects present in order to habituate squirrels to the new objects around the bait station. Each squirrel in our dataset was tested only once with the strike simulating device to control for prior exposure to the device. Sample videos of our trials can be viewed as Supplementary Material (Supplementary Video S1 and Video S2), and on our lab YouTube channel (<https://www.youtube.com/user/rulonclark>, last accessed September 18, 2014).

Data quantification

For each trial, we determined whether the squirrel tail flagged on approaching its bait station before the launch of the strike-simulating device. We also quantified the rate of tail flagging by counting the number of tail flag bouts that occurred in the minute before the launch of the device.

We used the software Premiere Pro (Adobe Systems Inc., San Jose, CA) to quantify from video both the speed of each squirrel's reaction time and their body displacement time in response to the simulated strike. We defined reaction time as the time from the first visible movement of the cork out of the PVC pipe to the first body movement made by the squirrel, and we defined body displacement time as the time it took the squirrel to fully displace its body from the position it was in before the spring was launched. Reaction time and body displacement time were not correlated (Pearson correlation: $r = 0.101$, $N = 64$, $P = 0.427$).

In addition, we quantified 2 distinct flee modalities that squirrels used to evade the simulated strikes: evasive leap, a vertical or sideways jump in the air with all 4 feet off the ground while swinging the tail, or scramble, during which squirrels turn around and run without leaping into the air (Figure 1A, Supplementary Video S2). Evasive leaps were qualitatively similar to the escape maneuvers we and others have observed squirrels use in response to free-ranging rattlesnake strikes (Hennessy and Owings 1988; Goldthwaite et al. 1990; Barbour and Clark 2012; Supplementary Video S3).

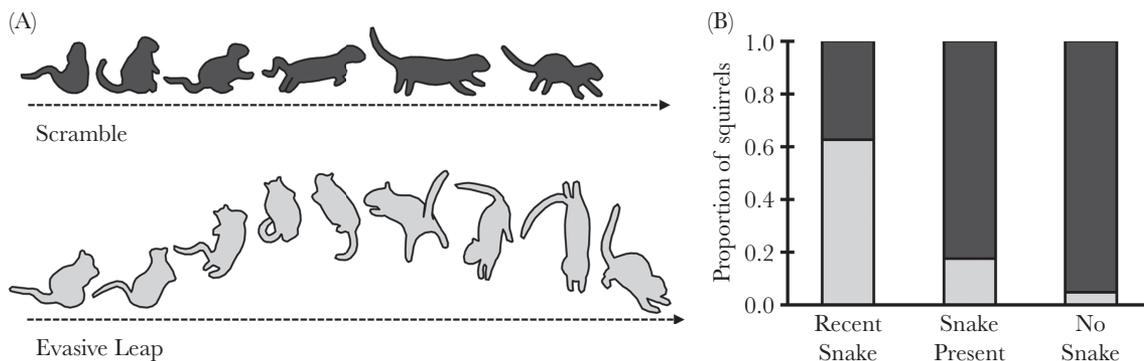


Figure 1

Two flee modalities squirrels used to evade simulated rattlesnake strikes. (A) Typical squirrel body positions from recording frames demonstrating the 2 distinct flee modalities. Squirrels in the first frame are in a bipedal foraging body position before the simulated attack, and have initiated an evasive response to the attack by frame 2. Dashed arrows indicate the direction the squirrel fled during its escape. (B) Proportion of squirrels that exhibited different flee modalities under the 3 experimental treatments. Dark gray bars indicate squirrels that utilized the scramble flee modality; Light gray bars indicate squirrels that utilized the evasive leap flee modality.

Statistical analyses

Over the course of two 8-week summer field seasons, we performed 22 Snake Present, 23 Recent Snake, and 21 No Snake trials. All statistical analyses were performed using SYSTAT 12.0 software (SPSS Inc., Chicago, IL). We used a likelihood ratio chi-square test to examine among-treatment differences in the proportion of squirrels that tail flagged. Additionally, we tested whether tail flagging rate of individuals that exhibited flagging differed between treatments by only examining data associated with positive tail flag occurrence (data associated with non-tail-flaggers were removed). We also removed data associated with the No Snake treatment because only 2 squirrels tail flagged under this treatment. We square-root transformed tail flagging rate to meet the assumption of normality and ran a 2-way *t*-test on the transformed variable to determine whether tail flagging rate differed between squirrels in the Snake Present and Recent Snake treatments.

We examined squirrel reaction and body displacement times using a general linear model with a Gaussian distribution that included 3 independent variables we determined a priori while designing the experiment: treatment, tail flagging occurrence (yes or no), and tail flagging rate (bouts/min). We included an additional variable, level of awareness, in our model a posteriori because previous studies have found that head position and/or food distraction can affect animal responses to predators (Lima and Bednekoff 1999; Stankowich and Blumstein 2005; Avilés and Bednekoff 2007). We created 3 levels of awareness that progress from most aware to least aware based on previous vigilance research: 1) head up not feeding, 2) head up feeding, and 3) head down feeding. We determined that a squirrel was feeding if it was actively handling and chewing sunflower seed. We did not have any squirrels that were not feeding while their head was down. For these general linear models (GLMs), we log transformed both reaction time and body displacement time to meet the assumption of model residual normality and assessed significance at $\alpha \leq 0.05$. For our analysis on reaction time, we excluded 4 outliers that had negative reaction times (i.e., they responded slightly before the cork visibly left the PVC pipe). We assume these individuals reacted to stimuli preceding the cork exiting the pipe, such as the movement of the observer cutting the fishing line or to the fishing line moving through the air after it had been cut. Because we could not see these events on our recordings we did not have a visible starting point for reaction time comparisons. We conducted logistic regression to determine which of the 4 independent variables affected flee modality. For the independent variables that had more than 3 categories (i.e., treatment and awareness level), we used multiple Wald's tests to examine all pairwise comparisons and included a Bonferroni correction to adjust the α level of these multiple comparisons. We looked for significant interactions in all of these models, but found none.

We could not control for the effect of snake size in this study because even with a large search effort, we were unable to collect a large number of similar-sized snakes in the field. Although we attempted to always use large adult snakes, we had to occasionally conduct trials with smaller adult snakes. Because snakes are gape-limited predators, head size (which is correlated with body size) affects the size of prey they are able to consume. There is a linear relationship between mass of the prey item and mass of the rattlesnake, but rattlesnakes typically do not consume prey more than 50% of their body mass (Shine 1991; Clark 2002; Glaudas et al. 2008). At our site, we have only observed large snakes of 600 g or more to eat adult ground squirrels. Compared with

small snakes, large snakes may prey on adult squirrels more often than squirrel pups (and be viewed as a more dangerous predator) so we tested whether snake size influenced squirrel responses in our experiment. We categorized the snakes we used as either large (>500 g, $n = 7$ snakes) or small (200–500 g, $n = 4$ snakes). We used 500 g as our cutoff instead of 600 g because 1 snake had a mass of 585 g which we estimated as large enough to consume adult squirrels. All other snakes in the small category were less than 400 g, but still as large as free ranging snakes we regularly observed hunting within squirrel colonies. Thus, snakes in our small category were large enough to consume young squirrels, and potentially dangerous to adults. We used GLMs to examine the effect of snake size on reaction time and body displacement time, and logistic regression to examine the effect of snake size on flee modality. We excluded data from the No Snake treatment, and included tail flag occurrence, tail flag rate, and treatment as additional factors in these models. We also looked for any significant interactions between factors. We found a strong effect of snake size on reaction time ($F_{1,38} = 9.20$, $P = 0.004$). Hence, we excluded data associated with squirrels that were presented small snakes (Recent Snake, $N = 9$; Snake Present, $N = 6$) and re-ran our analysis on reaction time using a reduced model. Although we found no effect of snake size on body displacement time and flee modality ($F_{1,42} = 0.002$, $P = 0.968$ and $\zeta = -0.45$, $P = 0.652$, respectively), we also ran reduced models for these variables excluding data associated with small snake presentations. We did this because snake size affected tail flagging occurrence with a higher proportion of squirrels tail flagging when presented with large snakes compared with small snakes ($\chi^2_1 = 7.78$, $P = 0.005$). We also ran full and reduced models for all dependent variables because reaction time and body displacement time were related to flee modality; squirrels that used an evasive leap reacted faster ($t_{65} = -2.12$, $P = 0.038$) and displaced their bodies more quickly ($t_{66} = -6.55$, $P < 0.001$) than squirrels that scrambled. We report the results of both the full and reduced models (Table 1).

RESULTS

Tail flagging occurrence and rate

Squirrels' use of tail flagging significantly differed between treatments ($\chi^2_2 = 13.69$, $P = 0.001$, Figure 2), such that only 9.5% of squirrels tail flagged during the No Snake treatment whereas 60.9% and 37.5% tail flagged during the Snake Present and Recent Snake treatments respectively (Supplementary Video S1). We found that when squirrels tail flagged, the rate of tail flagging did not differ between the Snake Present or Recent Snake treatments ($t_{21} = -1.23$, $P = 0.232$).

Reaction time

The GLM containing the full dataset showed that tail flagging occurrence significantly affected reaction time (Table 1). However, when the effect of snake size is removed, treatment becomes the only significant factor affecting reaction time (Figure 3A), whereas all other factors remained non-significant (Table 1). Post hoc comparisons using Fisher's LSD test revealed that reaction time in the Recent Snake treatment was significantly quicker than reaction time in the No Snake treatment ($P = 0.001$), and in Snake Present treatment ($P = 0.027$). Reaction time did not differ between the Snake Present and No Snake treatments ($P = 0.437$, Figure 3A).

Table 1
Results of the full and reduced models that examined factors affecting squirrel responses to simulated rattlesnake strikes

Reaction time ^a	Factor	df	Full model (<i>N</i> = 64)		Reduced model (<i>N</i> = 49)	
			<i>F</i> -ratio	<i>P</i> -value	<i>F</i> -ratio	<i>P</i> -value
	Treatment	2	2.74	0.073	6.14	0.005
	Tail flag occurrence	1	4.78	0.033	0.62	0.436
	Tail flag rate	1	0.00	0.970	0.00	0.963
	Awareness	2	0.53	0.589	0.37	0.692
Body displacement time ^a	Factor	df	Full model (<i>N</i> = 68)		Reduced model (<i>N</i> = 53)	
			<i>F</i> -ratio	<i>P</i> -value	<i>F</i> -ratio	<i>P</i> -value
	Treatment	2	6.27	0.003	6.63	0.003
	Tail flag occurrence	1	0.07	0.789	0.43	0.517
	Tail flag rate	1	5.32	0.025	7.10	0.011
	Awareness	2	1.58	0.214	2.12	0.131
Flee modality ^b	Comparisons		Full model (<i>N</i> = 68)		Reduced model (<i>N</i> = 53)	
			ζ -score	<i>P</i> -value	ζ -score	<i>P</i> -value
	Snake Present–No Snake		0.77	0.444	0.49	0.627
	Recent Snake–No Snake		3.18	0.001	2.82	0.005
	Recent Snake–Snake Present		3.03	0.002	2.51	0.012
	HDF–HUN		0.23	0.816	-0.01	0.992
	HUF–HUN		1.24	0.214	1.26	0.208
	HUF–HDF		1.27	0.205	1.38	0.168
	Tail flag yes/no		0.84	0.404	0.80	0.424
	Tail flag rate		1.96	0.049	1.98	0.048

P-values that were lower than the specified α -level are bolded. HDF = head down feeding, HUN = head up not feeding, HUF = head up feeding.

^a $\alpha \leq 0.05$.

^b $\alpha \leq 0.0167$ with Bonferroni correction for categories with multiple comparisons (treatment and level of awareness).

Body displacement time

Treatment significantly affected body displacement time in both the full and reduced models (Table 1). Post hoc comparisons using Fisher's LSD test revealed that squirrels displaced their bodies more quickly under the Recent Snake treatment compared with the No Snake treatment (full model: $P = 0.013$; reduced model: $P = 0.009$), and compared with the Snake Present treatment (full model: $P = 0.002$; reduced model: $P = 0.001$). There was no difference in body displacement times between the Snake Present and No Snake treatments (full model: $P = 0.501$; reduced model: $P = 0.495$; Figure 3B). Body displacement time was also negatively related to tail flagging rate (Table 1, Figure 4). Tail flagging occurrence and level of awareness did not affect body displacement time in either model (Table 1).

Flee modality

The full and reduced models both showed that squirrels in the Recent Snake treatment were more likely to use an evasive leap than a scramble compared with the Snake Present and to the No Snake treatment (Table 1, Figure 1B). Squirrels in the Snake Present treatment were not more likely to use an evasive leap than scramble compared with squirrels in the No Snake treatment (Table 1). Squirrels with higher tail flagging rates were more likely to leap than scramble (Table 1, Figure 5). Tail flagging occurrence did not affect flee modality (Table 1); the

proportion of non-tail-flaggers that performed evasive leaps was similar to that of tail-flaggers (27.9% and 32.0%, respectively). Level of awareness was also not associated with flee modality (Table 1).

DISCUSSION

In this experiment, we were able to use simulated snake strikes to examine the effectiveness of strike avoidance behavior of squirrels in different states of vigilance. As has been seen in past studies, we found that squirrels not only tail flagged directly toward snakes, but also tail flagged in areas where they had recently encountered a snake (within the previous hour). Squirrels that had recently encountered snakes at that site had quicker reaction and body displacement times, and were more likely to employ evasive aerial leaps in response to simulated strikes (Figure 3A). The rate of tail flagging also affected squirrels' body displacement time and flee modality. As strike velocities of rattlesnakes may reach up to 4.5 m/s (Cundall and Beaupre 2001), it could take snakes less than 70 ms to strike prey 30 cm away. Thus, even the small differences we recorded in squirrels' reaction and body displacement times among treatments could be the difference between life and death.

Our findings indicate that conspicuous prey displays in the squirrel/rattlesnake system that are given in the absence of snakes are not necessarily dishonest or mistaken signals of predator perception, but rather can be honest signals of vigilance (or readiness for

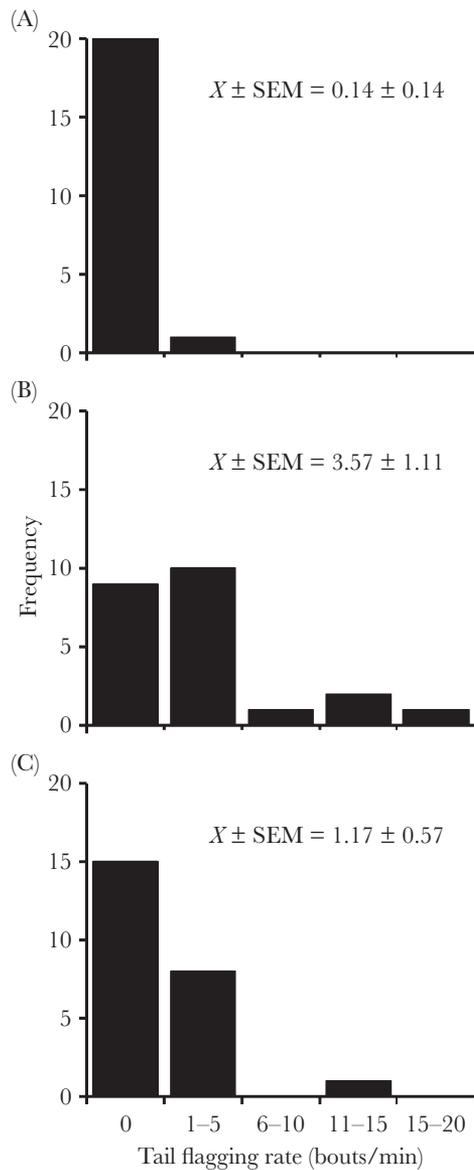


Figure 2

Histograms showing tail flagging rates of all squirrels in the 3 treatments: (A) No Snake, (B) Snake Present, and (C) Recent Snake. Mean (\pm SEM) tail flagging rate is also shown for each treatment.

a predatory attack). Many past studies have proposed a vigilance advertisement function for conspicuous animal displays emitted in the absence of predators (Hersek and Owings 1993; Murphy 2006; Randler 2006; Avellis 2011). However, the conclusions from these studies are mainly based on observations that animals signal most often during situations when they tend to also exhibit increased vigilance. For instance, tail wagging in wagtails positively correlates with head-up scanning, but not with head-down pecking (Randler 2006); tail flicking in chaffinches occurs more in open than obstructed habitats (Jones and Whittingham 2008); and display rates in several species increase after playbacks of predator sounds (Randler 2007; Avellis 2011). Ours is the first study, to our knowledge, which experimentally tests the ability of signalers to avoid a surprise attack. Our findings also indicate that behaviors commonly used as correlates of vigilance (i.e., level of awareness in this study) were not predictive of squirrel responses to predatory attacks. Head

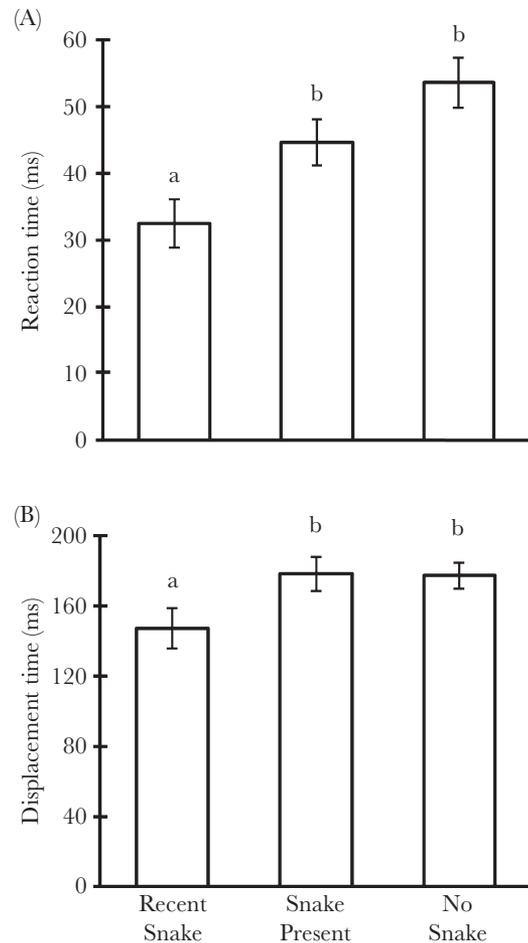


Figure 3

Squirrel reaction time and speed (body displacement time) under different treatments presenting simulated rattlesnake strikes in the reduced GLM analyses. (A) Mean (\pm SEM) reaction times of squirrels under the 3 experimental treatments. (B) Mean (\pm SEM) body displacement times of squirrels under the 3 experimental treatments. Letters over the bars indicate statistical significance at $\alpha \leq 0.05$.

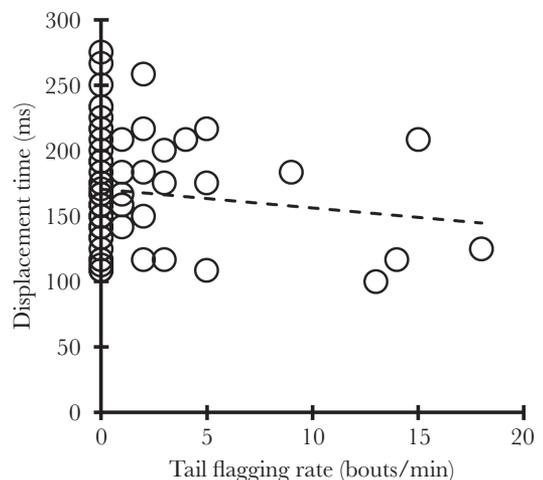


Figure 4

The relationship between body displacement time and rate of tail flagging. Squirrels with higher tail flagging rate moved more quickly when reacting to the strike simulating device (i.e., had lower body displacement time).

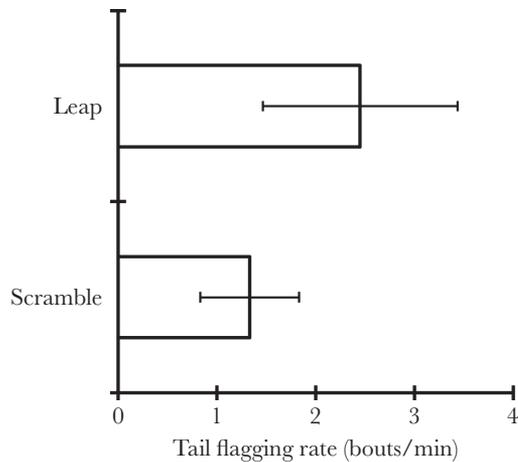


Figure 5

Tail flagging rates of squirrels exhibiting different flee modalities. Squirrels with higher tail flagging rates were more likely to perform evasive leaps.

position and feeding may not always be reliable indicators of vigilance (see Lima and Bednekoff 1999). In our system, prey appear to use specific evolved signals to advertise vigilance; such signals may be prevalent in many systems, given that prey often have incomplete knowledge regarding the presence of predators (Lima and Dill 1990; Sih 1992; Bergstrom and Lachmann 2001).

Although they tail flagged in both contexts, we found that a higher proportion of squirrels tail flagged when they actively confronted rattlesnakes than when they had recently perceived rattlesnakes. This result corroborates natural observations of squirrels interacting with visible rattlesnakes, which demonstrate that repeated tail flagging directed toward snakes likely functions to both alert the snake of detection and to enhance vigilance in nearby squirrels, thus further degrading the value of the snake's hunting area (Hersek and Owings 1993; Barbour and Clark 2012). As shown by Barbour and Clark (2012), this usually causes the snake to relocate away from the site of confrontation to an area containing uninformed prey. Tail flagging has also been shown to change with short-term motivational states (and is an inconsistent behavior across time) making it unlikely to be affected strongly by body condition or personality, which persist over longer time scales (Coss and Biardi 1997). Thus, tail flagging appears to function for both vigilance and perception advertisement, but does not necessarily indicate squirrel quality or identity. Some theoretical predictions concerning signaling behavior may be difficult to test in this system because of the multiple functions of tail flagging. For example, many aspects of our system fit the general framework of Bergstrom and Lachmann's (2001) game theory model of prey signaling; however, their model predicts that prey that are most certain of the presence of a predator should not always signal, because they would benefit most by simply avoiding the predator. This prediction is difficult to test in systems where the same signal that is used to advertise vigilance is also used to advertise awareness to the predator and predator presence to other potential prey.

Our results also indicate that not all individuals in a state of vigilance emit predator-directed signals (i.e., some non-tail-flagging squirrels initiated evasive leaps with fast body displacement times), and that signaling does not always indicate the readiness to quickly evade an attack; the relationship between tail flagging and response to simulated strikes was not perfect. Vigilant animals may refrain from signaling because of costs associated with signaling.

Although it is unlikely tail flagging incurs a large energetic cost, it probably increases the conspicuousness of squirrels to more visually-oriented predators such as raptors, coyotes, or foxes (Hanson and Coss 1997). This is the most likely reason why tail flagging is not ubiquitous (i.e., if there is no cost to tail flagging and it deters predators, squirrels should tail flag all the time; see Bergstrom and Lachmann 2001), and may explain why some squirrels refrained from tail flagging even in a heightened state of vigilance. A recent model demonstrates that individuals can differ in their communication strategies when signalers possess imperfect information about their own state and when signals are costly (Botero et al. 2010). Therefore, a range of alternative signaling strategies, from conservative signalers that pay low signaling costs to bluffing signalers that exaggerate their current state, can exist in a population. Our observations that some tail-flagging squirrels displayed weak evasive responses are consistent with this idea. However, because squirrels tail flagged more often in the Recent Snake treatment than the No Snake treatment, and tail flagging was generally associated with faster body displacement times during a simulated attack, we conclude that this signal can honestly indicate vigilance, and should act as a deterrent to snake attacks (as in Barbour and Clark 2012).

The evasive leap maneuver squirrels implement in response to rattlesnake strikes has been qualitatively described before (Hennessy and Owings 1988; Owings and Coss 2008; Barbour and Clark 2012, Supplementary Video S3), but ours is the first study we know of to record and quantify this behavior in detail. Although some species show no relationship between enhanced vigilance and flee behaviors (Adams et al. 2006; Boyer et al. 2006), we found that squirrels were more likely to use an evasive leap than a scramble when in a heightened state of vigilance induced by recently encountering a snake (Figure 3B). We believe that squirrels did not respond as strongly under the Snake Present treatment because they were actively monitoring the head of the live snake, making the launch of the strike-simulating device less mimetic of a strike (because it came from a different angle than the snake's head). Squirrels using the evasive leap flee modality had significantly quicker reaction and body displacement times than those using the scramble flee modality. The evasive leap into the air appears to quickly propel the body of the squirrel away from the vector of a snake strike (Supplementary Video S2). Once snakes initiate an attack, their ability to alter their strike trajectory is limited (Kardong and Bels 1998), and so squirrels likely benefit more from displacing their bodies vertically or horizontally than by trying to outdistance the strike by moving within the same plane as the strike trajectory (as seen with scrambles). Our recordings show that squirrels appear to use their tails to contort their bodies while leaping (Figure 3A, Supplementary Video S2). Additional studies on the kinematic and biomechanical aspects of the squirrel leaping maneuver are underway to advance our understanding of this behavior.

Our recordings also revealed extremely short reaction times (~40ms) for squirrels evading simulated strikes. These results suggest that responses to rattlesnake strikes may be associated with (or evolved from) a startle response pathway. Startle reflexes have extremely short response latencies, are invoked by evocative stimuli, and activate hundreds of muscles throughout the body to help animals avoid surprise attacks (Yeomans and Frankland 1996; Koch 1999; Yeomans et al. 2002). However, our measures of reaction time are not directly comparable to neurophysiological measures reported for laboratory animals (reviewed in Yeomans et al. 2002) because we did not directly measure muscle activation response latencies. We measured reaction time based on the first visible movement of our

device in the video recording. Several events occur in rapid succession just prior to this movement that could have also elicited squirrel responses: The monofilament line is severed by the observer, which releases tension on the spring, which uncoils audibly and pushes the cork forward. Thus, the reaction times we report are best taken as relative measures of reaction time useful for comparisons between individuals in our different treatments.

We suggest, though, that the stimuli used to evoke the squirrel evasive response is primarily acoustic instead of visual. The visual system responds via G-protein-coupled receptors, which act much slower than the mechanoreceptors of the acoustic startle response (Yeomans et al. 2002). Additionally, our observations from this and previous studies show that many squirrels react rapidly to strikes even when facing away from the attacker (Clark et al. 2012), which suggests that the sound of a snake strike may be more important in eliciting a response than the sight of the moving snake. The sound of a snake strike is key in strike avoidance in another small mammal, Merriam's kangaroo rat, *Dipodomys merriami*. Kangaroo rats with intact auditory systems exhibit an evasive leap to avoid rattlesnake strikes, but experimentally deafened rats do not exhibit this maneuver (Webster 1962). Our strike-simulating device produces a rush of air and a mechanical burst of sound from the uncoiling of the spring, but we do not know whether the intensity of sound affects squirrel responses. Further research is being conducted to test the importance of sound for squirrels in avoiding rattlesnake strikes.

To our surprise, squirrels' reaction time to the strike-simulating device was strongly affected by snake size, even though we always used adult rattlesnakes. Other studies have shown that squirrels modify their antisnake behaviors based on snake size (Rowe and Owings 1996; Swaisgood et al. 1999; Swaisgood et al. 2003), but these studies compare juvenile and adult rattlesnakes (snakes less than 150 g vs. more than 500 g). Juvenile rattlesnakes used in these studies would be physically unable to consume squirrels. Observations of rattlesnake feeding behavior at our site indicated that all of the snakes we used were large enough to consume squirrels. The greater strike distance, strike speed, and venom gland volume (Klauber 1972; Hayes 1991; Hayes and Mackessy 2010) of larger snakes could make them considerably more dangerous to squirrels than smaller snakes, even within the size range achieved by sexually mature adult snakes. Swaisgood et al. (2003) found that squirrel mothers tail flagged more after hearing rattling sounds from large adult rattlesnakes than from small juvenile rattlesnakes. Our study shows that even adult snakes may present variable risk for ground squirrels based on their size, and that future studies should control for the effect of snake size when using live tethered snakes as elicitors of antipredator responses.

CONCLUSION

In summary, we found that free-ranging ground squirrels responded to simulated rattlesnake strikes most strongly when they had recently encountered a rattlesnake but could no longer see the snake. Squirrels generally did not tail flag when they did not expect to encounter a snake. Tail flagging most strongly correlated with perception of a discovered rattlesnake, but squirrels also used this signal when returning to the location of a recently encountered snake. These results indicate that tail flagging can serve both a vigilance advertisement and a perception advertisement function in different contexts, and that the fear of attack from an undetected predator persists for some time after discovering a predator. This fear may create important fitness tradeoffs in squirrels and other prey species.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>.

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