How animals perceive the risk of predation can have implications on their ecology and behavior. Upon discovering predatory snakes, California ground squirrels (*Otospermophilus beecheyi*) display a series of stereotyped antisnake behavior consisting of elongated body postures, close-range inspection, tail-flagging, tail-piloerection, and substrate throwing. Following snake encounters, ground squirrels show prolonged states of heightened vigilance, which persist even in the absence of snakes. Research in other systems has shown that vigilance can enhance detection of predators, and that antipredator behaviors can decrease the likelihood of predation. Yet there is little experimental evidence demonstrating how predation risk is actually perceived by prey, and the degree to which it influences detection of predators. Most animals have more than one predator, hence there are many components of predation risk that prey can cue in on to inform themselves of potential predatory attacks (e.g. predator-specific microhabitats, time of activity, or hunting strategies). Research that develops an understanding of how predation risk is perceived by animals will provide significant insight into the nature of antipredator behavior.

Free-ranging adult ground squirrels from the Diablo Mountain Range in Santa Clara County, CA were first exposed to a live tethered rattlesnake (*Crotalus oreganus*), a rattlesnake model, or a novel object, and their antisnake behaviors were recorded. We found that squirrels exhibited enhanced vigilance only after presentations with the live snake. In a second experiment, squirrels were initially primed with a live rattlesnake to elicit heightened vigilance, and then were...
presented with a rattlesnake model or a novel object, and their antisnake behaviors were again recorded. We found that heightened vigilance significantly increased antisnake behavior toward both the rattlesnake model and the novel object (objects that were not treated as dangerous in the first experiment). Squirrels primed with rattlesnake interactions also treated the rattlesnake model as more dangerous than the novel object. These results indicate the cues that elicit antipredator behavior in prey can vary with their perception of risk, and that increased vigilance in ground squirrels may serve to generally increase sensitivity to objects that resemble snakes.

**INTRODUCTION**

Predation is among the strongest selective forces, and can profoundly affect the morphology, physiology, and behavior of prey (Brown et al. 1988–cite; Caro 2005 – cite). Predation can have both direct effects on communities by removing individuals from the population, as well as indirect effects, usually by altering the behavior or physiology of prey (Sih et al. 1985; Kotler et al. 1993). Indirect effects of predators can have unprecedented influence, altering habitat use, time of activity, mode of foraging, diet, mating, and life history (Milinski & Heller 1978; Lima, Valone, and Caraco 1985; Sih et al. 1985; Lima and Dill 1990; Magnhagen 1991; Kotler et al. 1993; Candolin 1998 – cite). Many of these behavioral effects stem from increased investment in antipredatory vigilance (Lima and Dill 1990; Caro 2005). By increasing vigilance behaviors, prey are more likely to avoid predators and/or alert others before the predator comes too close (Caro 2005). In a study involving wedge-capped capuchins, Robinson (1981 – cite) demonstrated that more vigilant females had an advantage in detecting ground predators over non-vigilant individuals (see also Baldellou and Henzi 1992 – cite; Cowlishaw 1997b – cite). Additionally, FitzGibbon (1989 – cite) reported that more vigilant Thomson’s gazelles were less susceptible to
predation because they tended to react more rapidly to attacks by cheetahs. Vigilance thus enhances predator detection and can provide earlier opportunities to implement effective avoidance mechanisms (Schooley, Sharpe and van Horne 1996 – cite). Although benefitting prey in detecting and avoiding predators, investment in vigilance comes as a tradeoff. Increased vigilance can negatively affect animal foraging rates (Lima and Dill 1990), sleep patterns (Gauthier-Clerc, Tamisier, and Cezilly (et al) 1998- cite), patch use (Werner et al. 1983 – cite; Brown, Laundré, and Gurung 1999 – cite; Caro 2005 - cite), and reproductive effort (Magnhagen 1991 – cite).

Prey increase vigilance when they expect to encounter predators; this occurs at “high-risk” times or locations (i.e. situations in which the likelihood of predation is elevated) (Caro 2005). Prey can alter their vigilance using social cues (Metcalfe 1984a – cite), environmental variables (Leger, Owings, and Coss 1983; G. Roberts 1983), and cues derived from the predator’s behavior (Scannell, Roberts, and Lazarus 2001 – cite; Caro 2005 - cite) to mount an appropriate antipredator response. For bonnet macaques, flight response of troop members to a leopard model was a significant predictor of the flight response of conspecific onlookers (Coss & Ramakrishnan 2000). Certain neurons in the macaque visual cortex are attuned to texture regularity (Tanaka, 1996), especially to an orderly arrangement of spots (Tanaka et al., 1991). Several studies utilizing predator models also show that specific morphological features, such as two facing eyes, are evocative stimuli to some animals (Scaife 1976; Hennig 1977; Coss 1978; Coss 1979). Brown et al. (1992) reported that moving leopard silhouettes elicited alarm calling in captive-born and wild-caught vervet monkeys, and California ground squirrels can cue in on snake scale patterns and respond appropriately with antisnake behavior (Coss and Owings 1977; Coss 1991). Such usage of invariant predator cues are important factors that influence an
animal’s vigilance, especially when those predator-like cues are ambiguous (Coss & Ramakrishnan 2000).

Predator recognition is of interest to behavioral ecologists because many, perhaps most, prey defenses first require discriminating harmless situations from dangerous ones. It is also important for prey to distinguish between high and low-risk circumstances to avoid predation and costly antipredator behaviors. The differentiation of distinct visual schemata (e.g. predators) involves the discrimination of particular features from background and attributing meaning to their invariant qualities (Coss and Goldthwaite 1995; Coss 1999). Cryptic predators, such as snakes, owls, and some fish, display complex patterning, coloration, and textures that putatively increase stealth. However, the invariant properties of these features still provide recognition cues that are used by prey to defeat crypsis (Coss 1991a, Coss 1999). How prey determine levels of predation risk when dealing with cryptic predators can be difficult to interpret, but critical to understanding which cues are used to initiate vigilance behavior. Yet, the indications that modify vigilance in prey of cryptic predators, and the extent to which vigilance alters their defensive behavior, remains underexamined.

California ground squirrels (Otospermophilus beecheyi) are ideal organisms for studying antipredatory vigilance because they are diurnal, have relatively small home ranges, and are readily observable in their habitats. These animals have a multitude of predators and can discriminate between different predator types (Hennessy & Owings 1978 – cite; Towers & Coss 1990). Rattlesnakes (Crotalus spp.) are responsible for high predation rates in many populations of ground-dwelling sciurids (Lindsdale 1946; Fitch 1948; Fitch 1949). Rattlesnakes are ambush predators, and hide and hunt in cover, waiting for their prey to come within strike range (Clark et al. 2012-cite). They are cryptic in both coloration and habits (Fitch 1949 – cite), and use this sit-
and-wait ambush hunting strategy along with rapid strikes to envenomate ground squirrels (Hennessy and Owings 1988, Goldthwaite et al. 1990) that come close. Rattlesnakes also occupy ground squirrel colonies in extremely high densities, leading to frequent natural interactions with ground squirrels (Linsdale 1946, Barbour and Clark 2012). Consequently, squirrels must deal with regular threat of predation from a cryptic predator that could be found in many microhabitats in their environment (Hennessy and Owings 1988, Towers and Coss 1990).

California ground squirrels have evolved an arsenal of behavioral defenses to deter predatory snakes (Pacific rattlesnakes, *Crotalus oreganus*, and gopher snakes, *Pituophis catenifer*). This host of “antisnake” behavior includes tail-flagging (repeatedly waving the tail from side-to-side), tail piloerection (conspicuous bristling of tail fur), substrate throwing, and close-range investigation of snakes (Owings and Coss 1977, Rowe and Owings 1978, Hennessy et al. 1981). Coss (1993) further defined ground squirrel vigilance behavior as an overall increase in the frequencies of these antisnake defenses (e.g. tail piloerection, tail-flagging, elongated postures, substrate throwing) and cautious inspection of known snake microhabitats. Following a recent interaction with a rattlesnake, ground squirrels conspicuously behave as if they expect to find another snake lying in wait (Coss 1993-cite). They frequently pause and adopt elongated postures to inspect potential hiding places of ambushing snakes, tail-flagging intermittently as their investigation progresses (Owings and Coss 1977). After encountering a rattlesnake, squirrels can maintain elevated vigilance of snakes even when not directly engaging them (Owings et al. 1986, Hersek and Owings 1993). This feature allowed us to experimentally include or remove vigilance to assess its influence on antisnake behavior.

We designed a series of experiments to test the hypothesis that increased vigilance following rattlesnake interactions facilitates heightened attention toward snake-like cues and concordantly
increases directed antisnake behavior. We first quantified the relative degree of antisnake behavior squirrels exhibited towards live northern Pacific rattlesnakes (*Crotalus oreganus*), plaster models of rattlesnakes, and novel objects. In a subsequent experiment, we initially “primed” ground squirrels with live rattlesnakes and then analyzed their ensuing antisnake responses towards rattlesnake models and novel objects. We predicted that if encounters with live rattlesnakes served to increase vigilance specifically toward snakes, squirrels would increase antisnake behavior towards rattlesnake models, but not novel objects. If snake encounters increased antipredatory vigilance in general, then we predicted squirrels would show increased antisnake behavior toward both snake models and novel objects.

METHODS

Study Site

This research was conducted at the Blue Oak Ranch Reserve (BORR), a University of California ecological reserve perched at ca. 760 m on the west slopes of the Diablo Mountain Range in northern Santa Clara County, California. The habitat is characterized by steep to moderate hills covered by a mixed oak woodland landscape. Initial surveys of the area confirmed dense populations of both northern Pacific rattlesnakes (*C. oreganus*) and California ground squirrels (*O. beecheyi*). Therefore, the squirrels used in this study were considered rattlesnake-adapted.

Ground Squirrel Subjects

We trapped squirrels haphazardly throughout the colony, resulting in a sample size of 22 female and 7 male adults. We used Tomahawk live traps baited with black oil sunflower seeds. All trapped squirrels were measured, weighed, sexed, ear tagged, and dye marked with Nyanzol
while under Ketamine HCl anesthesia (40mg/kg injected intramuscularly). We released all squirrels where they were captured following recovery from anesthesia. Each squirrel was uniquely marked with dye to facilitate long-range identification of individuals with camera equipment.

*Rattlesnake Collection and Tethering*

Tethered *C. oreganus* were used to elicit ground squirrel antisnake behavior. We used 3 long-term captive rattlesnakes, and opportunistically caught 3 wild rattlesnakes to use for tethering. All snakes were large adult males (>500 g). Our tethering procedure followed Owings and Coss (1977) and Randall and Matocq (1997). Briefly, snakes were equipped with three removable bands on their backs. Placed equidistant from one another, each band was made by wrapping the body of the snake with athletic tape and fixing a loop of string that could provide an attachment point for a tether line. Three tent stakes were outfitted with short monofilament tether lines from which the string on the bands could be fastened. After driving the stakes into the ground in a triangle-shape, we wrapped the snake around the three stakes, sequentially tying each string from its back onto the attachment points in the ground. This tethering procedure allowed us to experimentally place live rattlesnakes at specific locations near the home burrows of marked squirrels.

All snakes were kept at the BORR field station throughout the entire duration of the study. They were housed in glass terraria with either paper towel or newspaper substratum, and were given a hide box and water *ad libitum*. After the eight-week experimental period, long-term captive snakes remained in captivity, while recently-caught snakes from BORR were released at their place of capture.
Experimental Procedure

We established reliable “bait stations” across the field site where marked squirrels were seen actively foraging. We placed small amounts of black oil sunflower seeds at each bait station to habituate squirrels to return to that location to feed. After squirrels became habituated, we applied more bait, retreated to an observation point behind a camouflaged hunting blind (approximately 10 m from the bait station), and waited for a marked squirrel to approach. Once a focal squirrel approached the bait station, we filmed a baseline measurement of its behavior while feeding. During baselines, squirrels were allowed to feed on the bait undisturbed until they left. Baseline measurements were recorded to gather data on squirrel behavior before the influence of a stimulus presentation, and to ensure that the squirrel was not exhibiting vigilance indicative of a recent unaccounted rattlesnake interaction.

We conducted two main experiments on free-ranging squirrels, which will be referred to herein as “non-primed” and “snake-primed.” All treatments in both experiments were preceded by a recording of baseline behavior. The non-primed experiment was conducted to confirm that live rattlesnakes elicit the greatest antipredator response in ground squirrels, and to compare rattlesnake responses to those elicited from snake models and non-snake, novel objects. For this experiment, we presented one of three stimuli to focal squirrels returning to a bait station after a baseline: (1) a live tethered Pacific rattlesnake, (2) a rattlesnake model, or (3) a water bottle (non-snake, novel object). Rattlesnake models were used to provide cues representative of live snakes (e.g. snake scale pattern) in an effort to determine whether squirrels would respond to them with similar antisnake behaviors as live predators. Models were made out of plaster and painted to resemble real rattlesnakes, and were stored each night in terraria housing live captive rattlesnakes. We used a 1L sports water bottle as our novel object. Novel objects served as
innocuous stimuli, and allowed us to quantify antisnake behavior not attributed to the inherent perceptual elicitors of snakes and snake-like objects. Baseline recordings followed by a single stimulus presentation concluded the non-primed trials.

The snake-primed experiment was conducted to determine whether squirrels alter their sensitivity towards introduced objects (rattlesnake models and novel objects) after recent encounters with live rattlesnakes. During this experiment, initial baseline measurements were always followed by a live rattlesnake presentation. After the squirrel interacted with the rattlesnake and left the area, the snake was removed and replaced by either a rattlesnake model or novel object. The object was placed in a different location than the live snake, but within one meter of where the live snake was tethered. We did this to differentiate antisnake behaviors towards the objects from those directed towards the previous location of the snake. We filmed the returning squirrel’s interaction with the object.

An individual squirrel received only one of the two experiment types, and was presented with one treatment for any given 24 hr period (i.e. squirrels were re-tested under a different treatment only after 24 hours had elapsed). Since ground squirrels often encounter snakes daily, we were confident in the realism of the time frame used for treatment presentations. All trials were video recorded in the field from an observation blind using Sony® Handycam camcorders affixed to tripods. Treatments were conducted in a balanced order, and video data were later quantified for antisnake behavior.

Behavioral Measurements

We used video recordings to quantify ground squirrel behaviors in detail. Video decoding included transcribing narrated information and quantifying the following behavioral
measurements for statistical analyses: number of tail-flagging bouts, number of head-bobbing
acts, seconds spent investigating the stimulus (investigation time), number of substrate throws,
number of alarm calls, occurrence of tail piloerection (absent or present), and close proximity to
the snake or object (absent or present). Close proximity was defined as whether or not the
squirrel came within 30 cm of the snake or stimulus.

The various antisnake behaviors we used to measure ground squirrel vigilance followed the
original descriptions of Owings and Coss (1977). Although the head-bobbing behavior exhibited
during snake interactions has not been formally quantified in previous studies, it has been
described anecdotally. We quantified this behavior here because it was one of the most
consistent behaviors ground squirrels exhibited toward live rattlesnakes. We defined head-bobs
as the rapid, repeated lifting and lowering of the head in a side-to-side or up-and-down motion
while staring in the direction of the stimulus. Head-bobs were counted as bouts of bobbing
movements.

Statistical Analyses

We attempted to expose each individual squirrel to all treatments in each experiment (non-
primed and snake-primed), but because these squirrels were free-ranging, some individuals could
not be relocated after participating in experiments. Since some individual squirrels participated
in more than one treatment, we used generalized linear mixed models (GLMMs) to analyze the
effects of treatment on ground squirrel behavior. We used GLMMs fit by the Laplace
approximation as implemented in the lme4 package in R version 3.0.9. We included squirrel
identity as a random effect in all models, allowing individuals to have both random slopes and
intercepts.
Pilot trials examining ground squirrel antisnake behavior indicated that the most consistent behaviors displayed toward live snakes were tail flags and head bobs. Our counts of tail flags and head bobs were highly correlated ($r=0.92$), so rather than analyze these variables independently, we combined them into a single measure. Thus, “snake-directed behavior” in our models was defined as the summation of all tail flags and head bobs directed toward the stimulus.

For all response variables (snake-directed behavior, investigation time, tail piloerection, proximity) we evaluated the effect of treatment (type of stimulus presented) using GLMMs with treatment and squirrel sex as fixed factors. We used GLMMs with a Poisson distribution and log link function for data that were not overdispersed (i.e., when mean and variance were similar). We used lognormal Poisson GLMMs for overdispersed data by incorporating an additional observation level random effect (Elston et al. 2001, Bolker et al. 2009). Binomial GLMMs were used to model tail piloerection and close proximity to stimulus. We compared treatment levels in our models using Wald’s Z tests. In the non-primed experiments with three treatment levels (rattlesnake, rattlesnake model, and novel object), we used Tukey post hoc tests implemented in the multcomp package to conduct pairwise treatment comparisons.

Although alarm call vocalizations and substrate throwing are important antisnake behaviors (the latter being a prominent snake-directed defense in some ground squirrel populations) (Owings and Coss 1977, Rowe and Owings 1978, Loughry and McDonough 1989), our counts for these variables were extremely low. Across all trials, 13.8% of all squirrels alarm called, and only 10.8% threw substrate. Consequently, we did not analyze these behaviors statistically and instead focused on snake-directed behavior (the sum of tail-flags and head-bobs), investigation time, tail piloerection, and proximity to stimulus. Because we found no effect on close proximity in the
non-primed experiments, we omitted proximity from further analyses in the snake-primed experiments.

RESULTS

Non-Primed Experiments

We conducted 19 live rattlesnake trials, 21 rattlesnake model trials, and 19 novel object trials after filming baseline squirrel behavior. Squirrels displayed significantly greater snake-directed behavior towards live rattlesnakes than snake models ($Z=4.97, p<0.001$) or novel objects ($Z=5.87, p<0.001$), but no difference in this behavior existed between models and novel objects ($Z=1.42, p=0.331$) (See attached figures). Squirrels spent significantly more time investigating rattlesnakes than snake models ($Z=5.12, p<0.001$) or novel objects ($Z=5.32, p<0.001$), but showed no difference in investigation time between models and novel objects ($Z=1.38, p=0.346$). Live rattlesnakes elicited significantly more tail piloerection than snake models ($Z=2.70, p=0.018$) and novel objects ($Z=3.96, p<0.001$), but squirrels displayed only marginal differences in tail piloerection during snake model and novel object presentations ($Z=2.07, p=0.095$). Finally, squirrels were no more likely to closely approach a live rattlesnake than a snake model ($Z=0.385, p=0.700$) or novel object ($Z=0.773, p=0.439$).

Snake-Primed Experiments

The non-primed experiments showed that ground squirrels did not display significant differences in the way they responded to rattlesnake models and novel objects. To determine if this would
change for squirrels that had been “primed” with rattlesnakes, we conducted 19 rattlesnake model trials and 19 novel object trials on squirrels that had recently interacted with a live tethered rattlesnake. When comparing the responses of snake-primed squirrels to rattlesnake models and novel objects, snake-directed behavior was significantly greater towards rattlesnake models than novel objects (Z=3.13, p=0.002) (See attached figures). However, squirrels primed with live rattlesnakes did not spend more time investigating snake models over novel objects (Z=0.889, p=0.374), and did not show a significant difference in tail piloerection (Z=0.828, p=0.407).

Non-Primed and Snake-Primed Comparisons

To evaluate whether recent encounters with live rattlesnakes increased ground squirrel sensitivity to predator-like cues, we compared the antisnake behaviors of snake-primed (N=19) and non-primed (N=21) squirrels that were presented with rattlesnake models. Compared to non-primed groups, snake-primed squirrels significantly increased snake-directed behavior towards rattlesnake models (Z=2.54, p=0.011) (See attached figures). However, snake-primed squirrels did not increase time spent investigating rattlesnake models over non-primed squirrels (Z=1.58, p=0.114), and were not more likely to engage in tail piloerection (Z=1.42, p=0.156).

Finally, to determine whether recent encounters with live rattlesnakes increased responses towards novel objects, we compared the antisnake behaviors of snake-primed (N=19) and non-primed (N=19) squirrels that were presented with sports water bottles. Snake-primed squirrels
displayed significantly greater snake-directed behavior towards novel objects than non-primed squirrels \((Z = 2.36, p=0.018)\). Snake-primed squirrels also spent significantly more time investigating novel objects than non-primed squirrels \((Z=2.88, p=0.004)\), and significantly increased tail piloerection in the presence of novel objects \((Z=2.55, p=0.011)\).

**DISCUSSION**

When confronting predatory snakes, California ground squirrels display a battery of stereotyped antisnake behaviors consisting of tail-flags, head-bobs, tail piloerection, elongated postures, and cautious investigation \((\text{Owings and Coss 1977, Coss 1993; Coss 1999})\). Following rattlesnake encounters, ground squirrels remain in states of heightened vigilance that mediate the expression of such antisnake behavior \((\text{Rowe and Owings 1978, Rowe et al. 1986, Coss 1993})\). In this study, we quantified ground squirrel antisnake behavior towards standardized stimuli, and characterized how enhanced vigilance after rattlesnake encounters altered these responses. As we predicted, we found that encounters with live rattlesnakes significantly increased the vigilance of California ground squirrels and enhanced their antisnake responses to models that resemble rattlesnakes. However, contrary to our expectations, squirrels that had recently interacted with rattlesnakes also exhibited increased antisnake behavior toward innocuous objects that bear no resemblance to snakes.

Without the influence of a recent rattlesnake encounter, ground squirrels in the non-primed experiment exhibited very little antisnake behavior towards both the snake model and novel object compared to a live rattlesnake. Although the model was painted to resemble a real rattlesnake, had scale patterns, and was housed with a live snake to imbue it with snake scent, it did not elicit a significantly greater antisnake response than a novel object that had no
resemblance to a snake. In contrast, our snake-primed experiment showed that after a recent rattlesnake encounter, squirrels exhibited significantly greater antisnake behavior toward the snake model than the novel object. Thus, squirrels in heightened states of vigilance due to recent encounters with snakes become more sensitive to predator cues (e.g. scale patterns). In a low-risk environment, squirrels apparently require more than an immobile snake-like object to mount an antisnake response. However, these same cues can elicit a strong antisnake response when they perceive themselves to be in a high-risk environment (i.e., one where a predator may be hiding in ambush). Coss and Owings (1985-cite) reported that the perception of snake-like features (e.g. rounded stones, curled leaves, and sticks) can trigger vigilance behavior and tail-flagging in laboratory settings. Other studies have shown that microhabitat features indicative of high snake predation risk (e.g. bushy, occluded habitats) can modify predator avoidance behaviors in several rodent species (Kotler et al. 1993; Wasko et al. 2014). Our study provides direct evidence that the degree of expression of antisnake behavior is strongly dependent on the current motivational state of the squirrel.

Since ground squirrels live in heterogeneous habitats (e.g. elaborate burrow systems, tall grass, trees, rock outcrops), rattlesnakes are granted a myriad of potential ambush sites. As low-energy ectothermic predators, rattlesnakes can also occur at much higher densities (100 to 1000 times higher) than endothermic ground squirrel predators like coyotes, bobcats, and birds of prey (Nowak et al, 2008). At our field site, informal estimates indicate rattlesnake densities can exceed 30 rattlesnakes per hectare within the ground squirrel colony during the active foraging season (Clark, unpublished data). However, because the success of a rattlesnake’s hunting behavior relies primarily upon crypsis and ambush (Barbour and Clark, 2012, Clark et al. 2012 - cite, Hennessy and Owings 1988, Owings and Coss 2008), ground squirrels can remain safe so
long as they do not come within the effective strike range of a coiled snake (about 30 cm, Clark et al 2012). Thus, increasing vigilance and antisnake behaviors after encountering a rattlesnake should be an effective antipredator strategy by upregulating these costly behaviors only under high predation risk. Furthermore, ground squirrels must remain vigilant even after a snake has left a specific ambush site because rattlesnakes move frequently between different ambush locations within a few meters (Barbour and Clark 2012). Maintaining vigilance of snake-like cues after encountering a rattlesnake may facilitate fast detection of relocated snakes that remain close by. Even if squirrels are uncertain about the presence of a snake, increasing antisnake displays (i.e. tail flagging) can still dissuade rattlesnake attacks (Barbour and Clark 2012) by advertising the squirrel’s vigilance and readiness for an attack. In fact, a recent study has found that vigilant squirrels displaying tail-flags are better at avoiding simulated snake strikes than squirrels not displaying antisnake behaviors (Putman and Clark, in press).

Squirrels in the snake-primed experiment consistently exhibited significant increases in snake-directed behavior (tail-flags and head-bobs) towards both snake models and novel objects than squirrels in the non-primed experiment. Compared to non-primed squirrels, snake-primed squirrels also had significantly greater investigation times and levels of tail piloerection toward novel objects. This result was unexpected, as it was the only occurrence in which snake-primed squirrels significantly increased investigation time and tail piloerection. Tail piloerection has been used as a reliable measure of sympathetic nervous system arousal (Siegel and Skog, 1970; Fuchs et al., 1985; Rowe et al., 1986; Coss and Biardi 1997; Coss 1999), and is exhibited by other rodents under threat (Heynen et al. 1989). Cautious investigation of snakes also involves a high state of arousal and vigilance associated with regulation of distance (Coss 1999). It may be that rattlesnakes act as general anxiety-provoking stimuli for squirrels, providing context for the
perceived threat of novel objects in our experiments. Moreover, studies suggest that population differences in antisnake behavior reflect geographic disparities in predation regimes (Coss and Owings 1977, Coss et al 1993, Coss 1999). In a study by Coss (1999) comparing the responses of 31 geographically-distinct populations of California ground squirrels to live snakes, arousal (tail piloerection) and time spent facing the snake was uniformly lower for snake-selected squirrels than relax-selected populations. Attenuated responses to rattlesnakes by rattlesnake-adapted populations presumably reflect their experience with snakes in nature, as well as decisions made to minimize contact with rattlesnakes and the chance of envenomation (Coss et al 1993). Since the squirrels used in our experiments were rattlesnake-adapted, lower tail piloerection and investigation time for snake-primed squirrels presented with rattlesnake models likely parallel these observations.

It is important to know which cues initiate predator recognition and the response chosen by prey after assessment of those cues. Studies utilizing predator models have elucidated context-dependent antipredator behavior and are valuable tools to disseminating cues used in predator recognition (Curio 1975; Coss 1978b, 1979a; Altbacker and Csanyi 1990; Brown et al 1992; Coss & Ramakrishnan 2000). By using rattlesnake models in varying contexts, our findings underscore the importance of recent rattlesnake encounters in the recognition of snake-like features within the environment, and demonstrate the effect of enhanced vigilance on antisnake behavior. We propose that ground squirrels identify areas of increased predation risk based on the presence or absence of snake predators. Our results suggest that increasing antisnake responses once information on a snake’s presence has been acquired is a parsimonious antipredator strategy. By increasing antisnake responses only after encountering a predator, California ground squirrels may optimize usage of costly antipredator behavior. Furthermore, the
increase in vigilance following rattlesnake encounters we documented has important implications for the indirect effects of cryptic predators on prey species. Although rattlesnakes may not remove many ground squirrels directly, squirrels interact with rattlesnakes much more frequently than they do with other predators (Linsdale 1946; Fitch 1948). Though these interactions may not often result in direct predation (Putman et al., in prep), they do appear to impact the inherent tradeoff between vigilance and time spent on other activities. When moving through complex environments such as rock outcrops and burrow aggregations, ground squirrels reduce foraging and increase vigilance toward potential rattlesnakes by stopping to investigate microhabitats where snakes may hide in ambush (Leger et al. 1983). Although vigilance helps animals detect potential threats quickly (Baldellou and Henzi 1992 – cite; Cowlishaw 1997b; Caro 2005), dedication to vigilance also reduces time that can be allocated towards other biologically important behaviors such as feeding, mating, tending young, and forming shelter (Powell 1974; Leger et al. 1983; Lima et al 1985; Hennessy and Owings 1988). It is therefore important for prey animals to develop a balance between staying vigilant and engaging in other behaviors. In other predator-prey systems, the indirect effects of predators have been shown to have wide-ranging implications for community dynamics (Smith et al. 2000; White and Garrott 2005), and it is likely that the same effects occur in our system. Future studies should address the possible community-level indirect effects stemming from the ambush-foraging behaviors of rattlesnakes. In a similar vein, a follow-up study will address the same effects reported in this study with a different cryptic predator of the California ground squirrel, the Pacific gopher snake (*Pituophus catenifer*). Lastly, though we did not set out to address sex-mediated differences in vigilance and antisnake behavior, other studies should implement this factor in their analyses.
Literature Cited


Snake-Primed: Snake-Directed Behavior

- Model: Mean Snake-Directed Behavior
- Model-Primed: Mean Snake-Directed Behavior
- Novelty: Mean Snake-Directed Behavior
- Novelty-Primed: Mean Snake-Directed Behavior

Significance levels: p = 0.011, p = 0.002, p = 0.018