

1 **RATTLESNAKE ENCOUNTERS ENHANCE VIGILANCE BEHAVIOR OF**
2 **CALIFORNIA GROUND SQUIRRELS**

3 *(Otospermophilus beecheyi)*

4
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10 How animals perceive the risk of predation can have implications on their ecology and behavior.
11 Upon discovering predatory snakes, California ground squirrels (*Otospermophilus beecheyi*)
12 display a series of stereotyped antisnake behavior consisting of elongated body postures, close-
13 range inspection, tail-flagging, tail-piloerection, and substrate throwing. Following snake
14 encounters, ground squirrels show prolonged states of heightened vigilance, which persist even
15 in the absence of snakes. Research in other systems has shown that vigilance can enhance
16 detection of predators, and that antipredator behaviors can decrease the likelihood of predation.
17 Yet there is little experimental evidence demonstrating how predation risk is actually perceived
18 by prey, and the degree to which it influences detection of predators. Most animals have more
19 than one predator, hence there are many components of predation risk that prey can cue in on to
20 inform themselves of potential predatory attacks (e.g. predator-specific microhabitats, time of
21 activity, or hunting strategies). Research that develops an understanding of how predation risk is
22 perceived by animals will provide significant insight into the nature of antipredator behavior.
23 Free-ranging adult ground squirrels from the Diablo Mountain Range in Santa Clara County, CA
24 were first exposed to a live tethered rattlesnake (*Crotalus oreganus*), a rattlesnake model, or a
25 novel object, and their antisnake behaviors were recorded. We found that squirrels exhibited
26 enhanced vigilance only after presentations with the live snake. In a second experiment, squirrels
27 were initially primed with a live rattlesnake to elicit heightened vigilance, and then were

28 presented with a rattlesnake model or a novel object, and their antisnake behaviors were again
29 recorded. We found that heightened vigilance significantly increased antisnake behavior toward
30 both the rattlesnake model and the novel object (objects that were not treated as dangerous in the
31 first experiment). Squirrels primed with rattlesnake interactions also treated the rattlesnake
32 model as more dangerous than the novel object. These results indicate the cues that elicit
33 antipredator behavior in prey can vary with their perception of risk, and that increased vigilance
34 in ground squirrels may serve to generally increase sensitivity to objects that resemble snakes.

35

36 **INTRODUCTION**

37 Predation is among the strongest selective forces, and can profoundly affect the morphology,
38 physiology, and behavior of prey (Brown et al. 1988-cite; Caro 2005 – cite). Predation can have
39 both direct effects on communities by removing individuals from the population, as well as
40 indirect effects, usually by altering the behavior or physiology of prey (Sih et al. 1985; Kotler et
41 al. 1993). Indirect effects of predators can have unprecedented influence, altering habitat use,
42 time of activity, mode of foraging, diet, mating, and life history (Milinski & Heller 1978; Lima,
43 Valone, and Caraco 1985; Sih et al. 1985; Lima and Dill 1990; Magnhagen 1991; Kotler et al.
44 1993; Candolin 1998 – cite). Many of these behavioral effects stem from increased investment in
45 antipredatory vigilance (Lima and Dill 1990; Caro 2005). By increasing vigilance behaviors,
46 prey are more likely to avoid predators and/or alert others before the predator comes too close
47 (Caro 2005). In a study involving wedge-capped capuchins, Robinson (1981 – cite) demonstrated
48 that more vigilant females had an advantage in detecting ground predators over non-vigilant
49 individuals (see also Baldellou and Henzi 1992 – cite; Cowlshaw 1997b – cite). Additionally,
50 FitzGibbon (1989 – cite) reported that more vigilant Thomson's gazelles were less susceptible to

51 predation because they tended to react more rapidly to attacks by cheetahs. Vigilance thus
52 enhances predator detection and can provide earlier opportunities to implement effective
53 avoidance mechanisms (Schooley, Sharpe and van Horne 1996 – cite). Although benefitting prey
54 in detecting and avoiding predators, investment in vigilance comes as a tradeoff. Increased
55 vigilance can negatively affect animal foraging rates (Lima and Dill 1990), sleep patterns
56 (Gauthier-Clerc, Tamisier, and Cezilly (et al) 1998- cite), patch use (Werner et al. 1983 – cite;
57 Brown, Laundré, and Gurung 1999 – cite; Caro 2005 - cite), and reproductive effort (Magnhagen
58 1991 – cite).

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

74 animal's vigilance, especially when those predator-like cues are ambiguous (Coss &
75 Ramakrishnan 2000).

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

89 California ground squirrels (*Otospermophilus beecheyi*) are ideal organisms for studying
90 antipredatory vigilance because they are diurnal, have relatively small home ranges, and are
91 readily observable in their habitats. These animals have a multitude of predators and can
92 discriminate between different predator types (Hennessy & Owings 1978 – cite; Towers & Coss
93 1990). Rattlesnakes (*Crotalus* spp.) are responsible for high predation rates in many populations
94 of ground-dwelling sciurids (Lindsdale 1946; Fitch 1948; Fitch 1949). Rattlesnakes are ambush
95 predators, and hide and hunt in cover, waiting for their prey to come within strike range (Clark et
96 al. 2012-cite). They are cryptic in both coloration and habits (Fitch 1949 – cite), and use this sit-

97 and-wait ambush hunting strategy along with rapid strikes to envenomate ground squirrels
98 (Hennessy and Owings 1988, Goldthwaite et al. 1990) that come close. Rattlesnakes also occupy
99 ground squirrel colonies in extremely high densities, leading to frequent natural interactions with
100 ground squirrels (Linsdale 1946, Barbour and Clark 2012). Consequently, squirrels must deal
101 with regular threat of predation from a cryptic predator that could be found in many
102 microhabitats in their environment (Hennessy and Owings 1988, Towers and Coss 1990).

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

118 We designed a series of experiments to test the hypothesis that increased vigilance following
119 rattlesnake interactions facilitates heightened attention toward snake-like cues and concordantly

120 increases directed antisnake behavior. We first quantified the relative degree of antisnake
121 behavior squirrels exhibited towards live northern Pacific rattlesnakes (*Crotalus oreganus*),
122 plaster models of rattlesnakes, and novel objects. In a subsequent experiment, we initially
123 “primed” ground squirrels with live rattlesnakes and then analyzed their ensuing antisnake
124 responses towards rattlesnake models and novel objects. We predicted that if encounters with
125 live rattlesnakes served to increase vigilance specifically toward snakes, squirrels would increase
126 antisnake behavior towards rattlesnake models, but not novel objects. If snake encounters
127 increased antipredatory vigilance in general, then we predicted squirrels would show increased
128 antisnake behavior toward both snake models and novel objects.

129

130 **METHODS**

131 *Study Site*

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

138 *Ground Squirrel Subjects*

139 We trapped squirrels haphazardly throughout the colony, resulting in a sample size of 22 female
140 and 7 male adults. We used Tomahawk live traps baited with black oil sunflower seeds. All
141 trapped squirrels were measured, weighed, sexed, ear tagged, and dye marked with Nyanzol

142 while under Ketamine HCl anesthesia (40mg/kg injected intramuscularly). We released all
143 squirrels where they were captured following recovery from anesthesia. Each squirrel was
144 uniquely marked with dye to facilitate long-range identification of individuals with camera
145 equipment.

146 *Rattlesnake Collection and Tethering*

147 Tethered *C. oreganus* were used to elicit ground squirrel antisnake behavior. We used 3 long-
148 term captive rattlesnakes, and opportunistically caught 3 wild rattlesnakes to use for tethering.
149 All snakes were large adult males (>500 g). Our tethering procedure followed Owings and Coss
150 (1977) and Randall and Matocq (1997). Briefly, snakes were equipped with three removable
151 bands on their backs. Placed equidistant from one another, each band was made by wrapping the
152 body of the snake with athletic tape and fixing a loop of string that could provide an attachment
153 point for a tether line. Three tent stakes were outfitted with short monofilament tether lines from
154 which the string on the bands could be fastened. After driving the stakes into the ground in a
155 triangle-shape, we wrapped the snake around the three stakes, sequentially tying each string from
156 its back onto the attachment points in the ground. This tethering procedure allowed us to
157 experimentally place live rattlesnakes at specific locations near the home burrows of marked
158 squirrels.

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

164 *Experimental Procedure*

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

175 We conducted two main experiments on free-ranging squirrels, which will be referred to herein
176 as “non-primed” and “snake-primed.” All treatments in both experiments were preceded by a
177 recording of baseline behavior. The non-primed experiment was conducted to confirm that live
178 rattlesnakes elicit the greatest antipredator response in ground squirrels, and to compare
179 rattlesnake responses to those elicited from snake models and non-snake, novel objects. For this
180 experiment, we presented one of three stimuli to focal squirrels returning to a bait station after a
181 baseline: (1) a live tethered Pacific rattlesnake, (2) a rattlesnake model, or (3) a water bottle
182 (non-snake, novel object). Rattlesnake models were used to provide cues representative of live
183 snakes (e.g. snake scale pattern) in an effort to determine whether squirrels would respond to
184 them with similar antisnake behaviors as live predators. Models were made out of plaster and
185 painted to resemble real rattlesnakes, and were stored each night in terraria housing live captive
186 rattlesnakes. We used a 1L sports water bottle as our novel object. Novel objects served as

187 innocuous stimuli, and allowed us to quantify antisnake behavior not attributed to the inherent
188 perceptual elicitors of snakes and snake-like objects. Baseline recordings followed by a single
189 stimulus presentation concluded the non-primed trials.

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

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

206 *Behavioral Measurements*

207 We used video recordings to quantify ground squirrel behaviors in detail. Video decoding
208 included transcribing narrated information and quantifying the following behavioral

209 measurements for statistical analyses: number of tail-flagging bouts, number of head-bobbing
210 acts, seconds spent investigating the stimulus (investigation time), number of substrate throws,
211 number of alarm calls, occurrence of tail piloerection (absent or present), and close proximity to
212 the snake or object (absent or present). Close proximity was defined as whether or not the
213 squirrel came within 30 cm of the snake or stimulus.

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

222 *Statistical Analyses*

223 We attempted to expose each individual squirrel to all treatments in each experiment (non-
224 primed and snake-primed), but because these squirrels were free-ranging, some individuals could
225 not be relocated after participating in experiments. Since some individual squirrels participated
226 in more than one treatment, we used generalized linear mixed models (GLMMs) to analyze the
227 effects of treatment on ground squirrel behavior. We used GLMMs fit by the Laplace
228 approximation as implemented in the lme4 package in R version 3.0.9. We included squirrel
229 identity as a random effect in all models, allowing individuals to have both random slopes and
230 intercepts.

231 Pilot trials examining ground squirrel antisnake behavior indicated that the most consistent
232 behaviors displayed toward live snakes were tail flags and head bobs. Our counts of tail flags and
233 head bobs were highly correlated ($r=0.92$), so rather than analyze these variables independently,
234 we combined them into a single measure. Thus, “snake-directed behavior” in our models was
235 defined as the summation of all tail flags and head bobs directed toward the stimulus.

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

246 Although alarm call vocalizations and substrate throwing are important antisnake behaviors (the
247 latter being a prominent snake-directed defense in some ground squirrel populations) (Owings
248 and Coss 1977, Rowe and Owings 1978, Loughry and McDonough 1989), our counts for these
249 variables were extremely low. Across all trials, 13.8% of all squirrels alarm called, and only
250 10.8% threw substrate. Consequently, we did not analyze these behaviors statistically and instead
251 focused on snake-directed behavior (the sum of tail-flags and head-bobs), investigation time, tail
252 piloerection, and proximity to stimulus. Because we found no effect on close proximity in the

253 non-primed experiments, we omitted proximity from further analyses in the snake-primed
254 experiments.

255

256 **RESULTS**

257 *Non-Primed Experiments*

258

259 We conducted 19 live rattlesnake trials, 21 rattlesnake model trials, and 19 novel object trials
260 after filming baseline squirrel behavior. Squirrels displayed significantly greater snake-directed
261 behavior towards live rattlesnakes than snake models ($Z=4.97$, $p<0.001$) or novel objects
262 ($Z=5.87$, $p<0.001$), but no difference in this behavior existed between models and novel objects
263 ($Z=1.42$, $p=0.331$) (See attached figures). Squirrels spent significantly more time investigating
264 rattlesnakes than snake models ($Z=5.12$, $p<0.001$) or novel objects ($Z=5.32$, $p<0.001$), but
265 showed no difference in investigation time between models and novel objects ($Z=1.38$, $p=0.346$).
266 Live rattlesnakes elicited significantly more tail piloerection than snake models ($Z=2.70$,
267 $p=0.018$) and novel objects ($Z=3.96$, $p<0.001$), but squirrels displayed only marginal differences
268 in tail piloerection during snake model and novel object presentations ($Z=2.07$, $p=0.095$).
269 Finally, squirrels were no more likely to closely approach a live rattlesnake than a snake model
270 ($Z=0.385$, $p=0.700$) or novel object ($Z=0.773$, $p=0.439$).

271

272 *Snake-Primed Experiments*

273

274 The non-primed experiments showed that ground squirrels did not display significant differences
275 in the way they responded to rattlesnake models and novel objects. To determine if this would

276 change for squirrels that had been “primed” with rattlesnakes, we conducted 19 rattlesnake
277 model trials and 19 novel object trials on squirrels that had recently interacted with a live
278 tethered rattlesnake. When comparing the responses of snake-primed squirrels to rattlesnake
279 models and novel objects, snake-directed behavior was significantly greater towards rattlesnake
280 models than novel objects ($Z=3.13$, $p=0.002$) (See attached figures). However, squirrels primed
281 with live rattlesnakes did not spend more time investigating snake models over novel objects
282 ($Z=0.889$, $p=0.374$), and did not show a significant difference in tail piloerection ($Z=0.828$,
283 $p=0.407$).

284

285

286 *Non-Primed and Snake-Primed Comparisons*

287

288 To evaluate whether recent encounters with live rattlesnakes increased ground squirrel sensitivity
289 to predator-like cues, we compared the antisnake behaviors of snake-primed ($N=19$) and non-
290 primed ($N=21$) squirrels that were presented with rattlesnake models. Compared to non-primed
291 groups, snake-primed squirrels significantly increased snake-directed behavior towards
292 rattlesnake models ($Z=2.54$, $p=0.011$) (See attached figures). However, snake-primed squirrels
293 did not increase time spent investigating rattlesnake models over non-primed squirrels ($Z=1.58$,
294 $p=0.114$), and were not more likely to engage in tail piloerection ($Z=1.42$, $p=0.156$).

295

296 Finally, to determine whether recent encounters with live rattlesnakes increased responses
297 towards novel objects, we compared the antisnake behaviors of snake-primed ($N=19$) and non-
298 primed ($N=19$) squirrels that were presented with sports water bottles. Snake-primed squirrels

299 displayed significantly greater snake-directed behavior towards novel objects than non-primed
300 squirrels ($Z = 2.36$, $p=0.018$). Snake-primed squirrels also spent significantly more time
301 investigating novel objects than non-primed squirrels ($Z=2.88$, $p=0.004$), and significantly
302 increased tail piloerection in the presence of novel objects ($Z=2.55$, $p=0.011$).

303

304 **DISCUSSION**

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

317 Without the influence of a recent rattlesnake encounter, ground squirrels in the non-primed
318 experiment exhibited very little antisnake behavior towards both the snake model and novel
319 object compared to a live rattlesnake. Although the model was painted to resemble a real
320 rattlesnake, had scale patterns, and was housed with a live snake to imbue it with snake scent, it
321 did not elicit a significantly greater antisnake response than a novel object that had no

322 resemblance to a snake. In contrast, our snake-primed experiment showed that after a recent
323 rattlesnake encounter, squirrels exhibited significantly greater antisnake behavior toward the
324 snake model than the novel object. Thus, squirrels in heightened states of vigilance due to recent
325 encounters with snakes become more sensitive to predator cues (e.g. scale patterns). In a low-risk
326 environment, squirrels apparently require more than an immobile snake-like object to mount an
327 antisnake response. However, these same cues can elicit a strong antisnake response when they
328 perceive themselves to be in a high-risk environment (i.e., one where a predator may be hiding in
329 ambush). Coss and Owings (1985-cite) reported that the perception of snake-like features (e.g.
330 rounded stones, curled leaves, and sticks) can trigger vigilance behavior and tail-flagging in
331 laboratory settings. Other studies have shown that microhabitat features indicative of high snake
332 predation risk (e.g. bushy, occluded habitats) can modify predator avoidance behaviors in several
333 rodent species (Kotler et al. 1993; Wasko et al. 2014). Our study provides direct evidence that
334 the degree of expression of antisnake behavior is strongly dependent on the current motivational
335 state of the squirrel.

336 Since ground squirrels live in heterogeneous habitats (e.g. elaborate burrow systems, tall grass,
337 trees, rock outcrops), rattlesnakes are granted a myriad of potential ambush sites. As low-energy
338 ectothermic predators, rattlesnakes can also occur at much higher densities (100 to 1000 times
339 higher) than endothermic ground squirrel predators like coyotes, bobcats, and birds of prey
340 (Nowak et al, 2008). At our field site, informal estimates indicate rattlesnake densities can
341 exceed 30 rattlesnakes per hectare within the ground squirrel colony during the active foraging
342 season (Clark, unpublished data). However, because the success of a rattlesnake's hunting
343 behavior relies primarily upon crypsis and ambush (Barbour and Clark, 2012, Clark et al. 2012 -
344 cite, Hennessy and Owings 1988, Owings and Coss 2008), ground squirrels can remain safe so

345 long as they do not come within the effective strike range of a coiled snake (about 30 cm, Clark
346 et al 2012). Thus, increasing vigilance and antisnake behaviors after encountering a rattlesnake
347 should be an effective antipredator strategy by upregulating these costly behaviors only under
348 high predation risk. Furthermore, ground squirrels must remain vigilant even after a snake has
349 left a specific ambush site because rattlesnakes move frequently between different ambush
350 locations within a few meters (Barbour and Clark 2012). Maintaining vigilance of snake-like
351 cues after encountering a rattlesnake may facilitate fast detection of relocated snakes that remain
352 close by. Even if squirrels are uncertain about the presence of a snake, increasing antisnake
353 displays (i.e. tail flagging) can still dissuade rattlesnake attacks (Barbour and Clark 2012) by
354 advertising the squirrel's vigilance and readiness for an attack. In fact, a recent study has found
355 that vigilant squirrels displaying tail-flags are better at avoiding simulated snake strikes than
356 squirrels not displaying antisnake behaviors (Putman and Clark, in press).

357 Squirrels in the snake-primed experiment consistently exhibited significant increases in snake-
358 directed behavior (tail-flags and head-bobs) towards both snake models and novel objects than
359 squirrels in the non-primed experiment. Compared to non-primed squirrels, snake-primed
360 squirrels also had significantly greater investigation times and levels of tail piloerection toward
361 novel objects. This result was unexpected, as it was the only occurrence in which snake-primed
362 squirrels significantly increased investigation time and tail piloerection. Tail piloerection has
363 been used as a reliable measure of sympathetic nervous system arousal (Siegel and Skog, 1970;
364 Fuchs et al., 1985; Rowe et al., 1986; Coss and Biardi 1997; Coss 1999), and is exhibited by
365 other rodents under threat (Heynen et al. 1989). Cautious investigation of snakes also involves a
366 high state of arousal and vigilance associated with regulation of distance (Coss 1999). It may be
367 that rattlesnakes act as general anxiety-provoking stimuli for squirrels, providing context for the

368 perceived threat of novel objects in our experiments. Moreover, studies suggest that population
369 differences in antislake behavior reflect geographic disparities in predation regimes (Coss and
370 Owings 1977, Coss et al 1993, Coss 1999). In a study by Coss (1999) comparing the responses
371 of 31 geographically-distinct populations of California ground squirrels to live snakes, arousal
372 (tail piloerection) and time spent facing the snake was uniformly lower for snake-selected
373 squirrels than relax-selected populations. Attenuated responses to rattlesnakes by rattlesnake-
374 adapted populations presumably reflect their experience with snakes in nature, as well as
375 decisions made to minimize contact with rattlesnakes and the chance of envenomation (Coss et al
376 1993). Since the squirrels used in our experiments were rattlesnake-adapted, lower tail
377 piloerection and investigation time for snake-primed squirrels presented with rattlesnake models
378 likely parallel these observations.

379 It is important to know which cues initiate predator recognition and the response chosen by prey
380 after assessment of those cues. Studies utilizing predator models have elucidated context-
381 dependent antipredator behavior and are valuable tools to disseminating cues used in predator
382 recognition (Curio 1975; Coss 1978b, 1979a; Altbacker and Csanyi 1990; Brown et al 1992;
383 Coss & Ramakrishnan 2000). By using rattlesnake models in varying contexts, our findings
384 underscore the importance of recent rattlesnake encounters in the recognition of snake-like
385 features within the environment, and demonstrate the effect of enhanced vigilance on antislake
386 behavior. We propose that ground squirrels identify areas of increased predation risk based on
387 the presence or absence of snake predators. Our results suggest that increasing antislake
388 responses once information on a snake's presence has been acquired is a parsimonious
389 antipredator strategy. By increasing antislake responses only after encountering a predator,
390 California ground squirrels may optimize usage of costly antipredator behavior. Furthermore, the

391 increase in vigilance following rattlesnake encounters we documented has important implications
392 for the indirect effects of cryptic predators on prey species. Although rattlesnakes may not
393 remove many ground squirrels directly, squirrels interact with rattlesnakes much more frequently
394 than they do with other predators (Linsdale 1946; Fitch 1948). Though these interactions may
395 not often result in direct predation (Putman et al., in prep), they do appear to impact the inherent
396 tradeoff between vigilance and time spent on other activities. When moving through complex
397 environments such as rock outcrops and burrow aggregations, ground squirrels reduce foraging
398 and increase vigilance toward potential rattlesnakes by stopping to investigate microhabitats
399 where snakes may hide in ambush (Leger et al. 1983). Although vigilance helps animals detect
400 potential threats quickly (Baldellou and Henzi 1992 – cite; Cowlshaw 1997b; Caro 2005),
401 dedication to vigilance also reduces time that can be allocated towards other biologically
402 important behaviors such as feeding, mating, tending young, and forming shelter (Powell 1974;
403 Leger et al. 1983; Lima et al 1985; Hennessy and Owings 1988). It is therefore important for
404 prey animals to develop a balance between staying vigilant and engaging in other behaviors. In
405 other predator-prey systems, the indirect effects of predators have been shown to have wide-
406 ranging implications for community dynamics (Smith et al. 2000; White and Garrott 2005), and
407 it is likely that the same effects occur in our system. Future studies should address the possible
408 community-level indirect effects stemming from the ambush-foraging behaviors of rattlesnakes.
409 In a similar vein, a follow-up study will address the same effects reported in this study with a
410 different cryptic predator of the California ground squirrel, the Pacific gopher snake (*Pituophis*
411 *catenifer*). Lastly, though we did not set out to address sex-mediated differences in vigilance and
412 antisnake behavior, other studies should implement this factor in their analyses.

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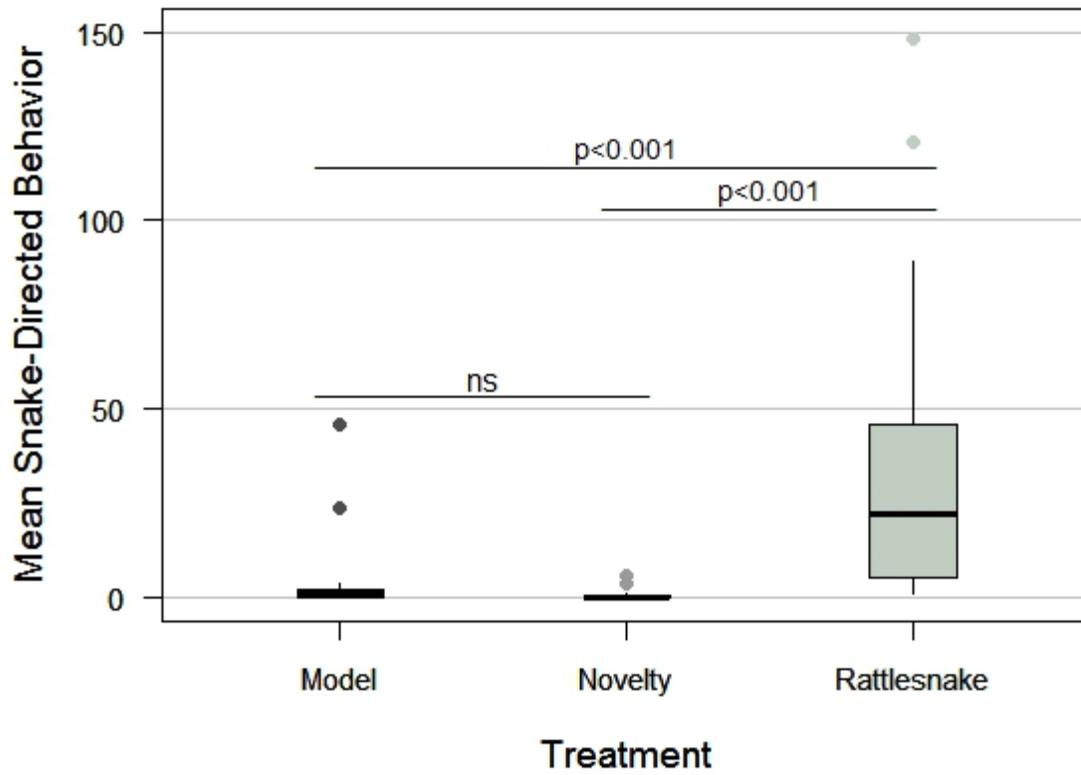
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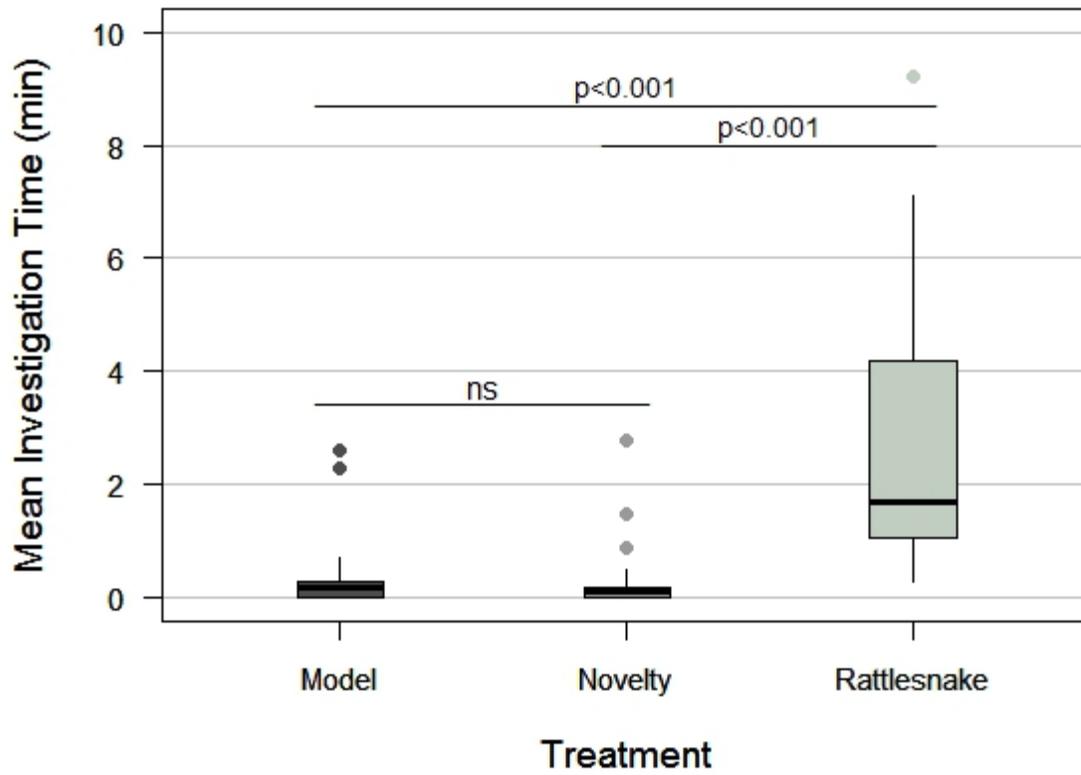
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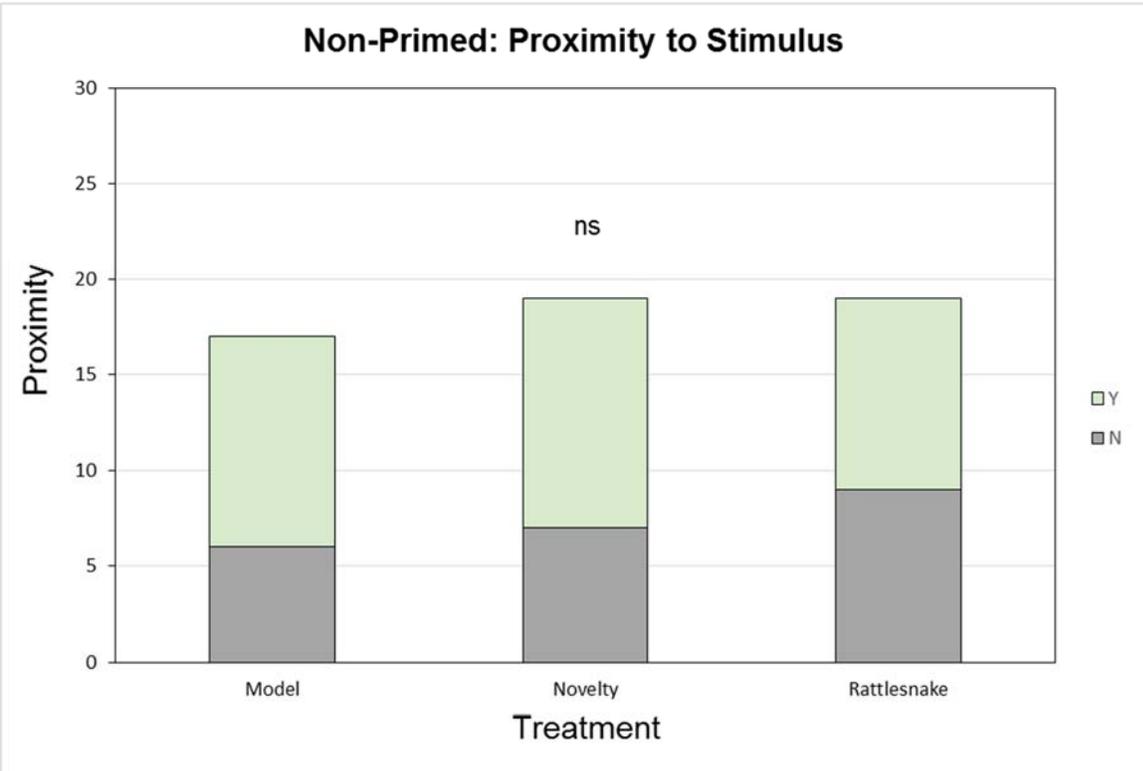
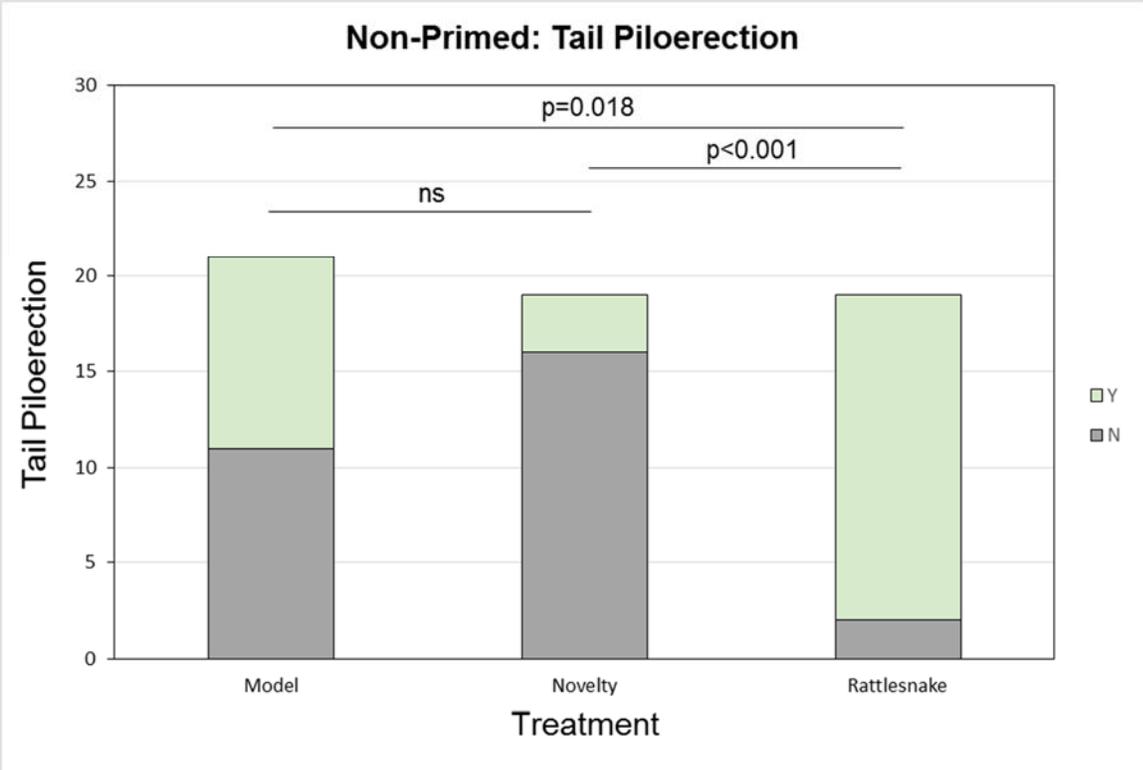
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Non-Primed: Snake-Directed Behavior

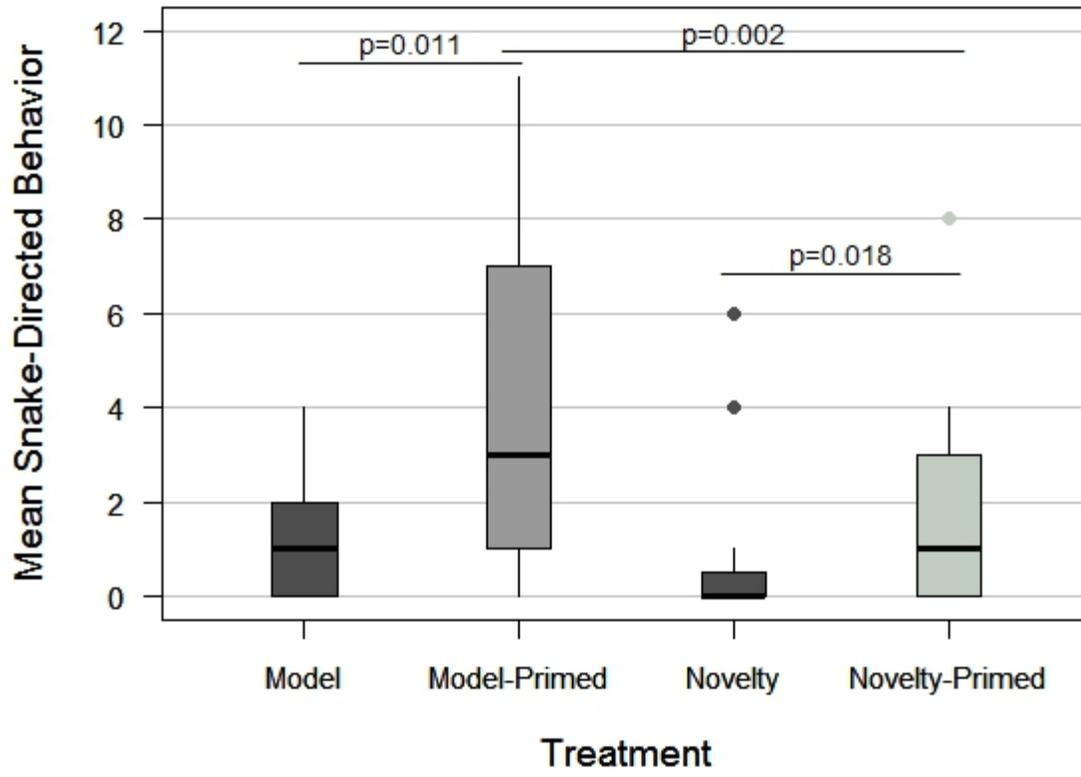


Non-Primed: Investigation Time

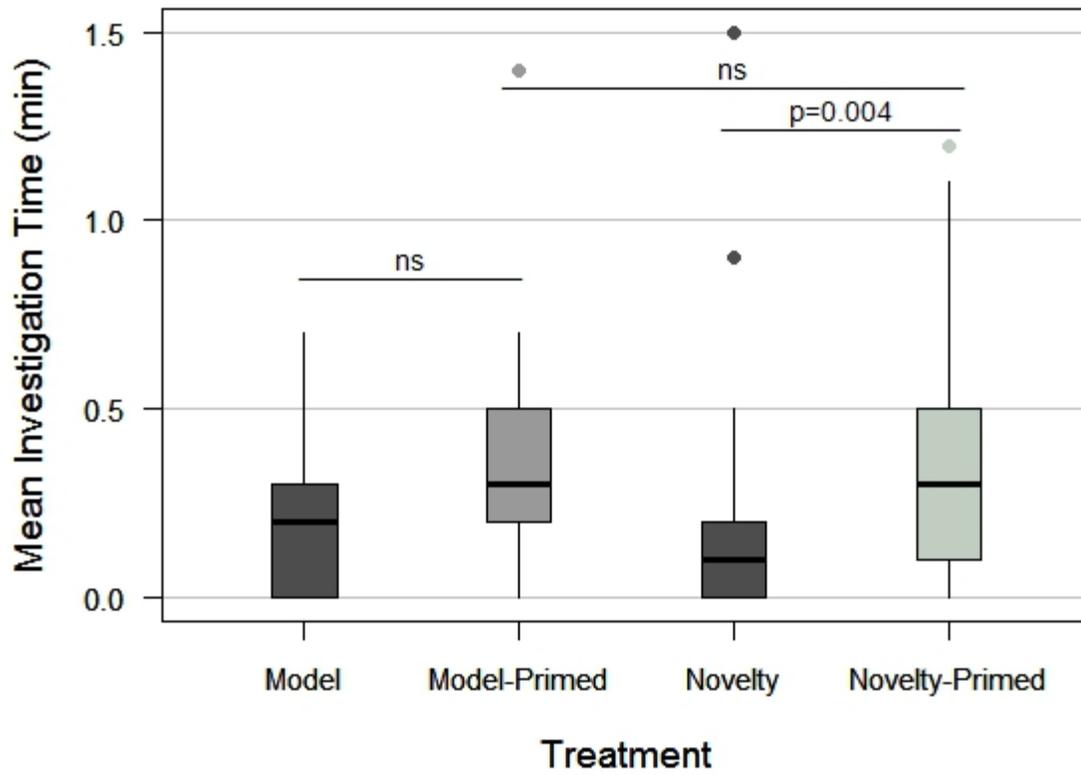




Snake-Primed: Snake-Directed Behavior



Snake-Primed: Investigation Time



Snake-Primed: Tail Piloerection

