

Ecological and hormonal correlates of antipredator behavior in adult Belding's ground squirrels (*Spermophilus beldingi*)

Jill M. Mateo

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Abstract Predator–prey relationships provide an excellent opportunity to study coevolved adaptations. Decades of theoretical and empirical research have illuminated the various behavioral adaptations exhibited by prey animals to avoid detection and capture, and recent work has begun to characterize physiological adaptations, such as immune reactions, metabolic changes, and hormonal responses to predators or their cues. A 2-year study quantified the activity budgets and antipredator responses of adult Belding's ground squirrels (*Spermophilus beldingi*) living in three different California habitats and likely experiencing different predation pressures. At one of these sites, which is visually closed and predators and escape burrows are difficult to see, animals responding to alarm calls remain alert longer and show more exaggerated responses than adults living in two populations that likely experience less intense predation pressure. They also spend more time alert and less time foraging than adults at the other two sites. A 4-year study using noninvasive fecal sampling of cortisol metabolites revealed that *S. beldingi* living in the closed site also have lower corticoid levels than adults at the other two sites. The lower corticoids likely reflect that predation risk at this closed site is predictable, and might allow animals to mount large acute cortisol responses, facilitating escape from predators and enhanced vigilance while also promoting glucose storage for the approaching hibernation. Collectively, these data demonstrate that local environments and

perceived predation risk influence not only foraging, vigilance, and antipredator behaviors, but adrenal functioning as well, which may be especially important for obligate hibernators that face competing demands on glucose storage and mobilization.

Keywords *Spermophilus* · Fecal cortisol · Antipredator strategies · Population differences

Introduction

Predator–prey interactions can have profound effects on the reproductive success, physiology and behavior of both hunters and the hunted. Coevolutionary arms races can favor the selection of strategies for both players to optimize their survival. Among prey species, these strategies include avoiding detection (e.g., cryptic coloration, circadian adjustment in activity, avoidance of certain microhabitats; Holmes 1984; Longland and Price 1991; Endler 1995; Ruxton et al. 2004; Ebensperger and Hurtado 2005), adjusting vigilance based on changes in risk (e.g., Goldsmith 1990; Longland and Price 1991; Schooley et al. 1996; Cavigelli 1999; reviewed in Lima 1998), and optimizing reactions when a predator is detected (e.g., Edmunds 1974; Magurran 1990; Mateo 1996; Mirza et al. 2006). In addition to behavioral adaptations, selection can favor physiological adaptations for avoiding or surviving predation attempts, such as modifications in metabolic rates, adrenal responses, immune functioning, and even levels of venom resistance (e.g., Poran et al. 1987; Hik et al. 2001; Wingfield 2004; Boonstra 2005). Finally, although often overlooked, variation in antipredator behavior can arise through proximate means as well because selection can favor behavioral plasticity within a species as a result of spatial and temporal changes

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J. M. Mateo (✉)
Institute for Mind and Biology
and the Department of Comparative Human Development,
The University of Chicago,
5730 S. Woodlawn Avenue,
Chicago, IL 60637, USA
e-mail: jmateo@uchicago.edu

in predation risk and developmental changes in vulnerability (e.g., Mateo and Holmes 1999; Griffin et al. 2001; Miner et al. 2005; Owings and Coss 2007). Unfortunately, little research has focused on counterstrategies of predators as a result of changes in prey adaptations (cf. Caro 1994; Cresswell and Quinn 2004; Mills et al. 2004).

When encountering a predator, a prey animal can exhibit stress responses, defined as the cumulative physiological reactions triggered by unpredictable events. Adrenal glucocorticoids protect the body during and after stress responses, in addition to regulating circadian rhythms and energy mobilization and storage. Stressor-induced functions of glucocorticoids include increasing available glucose, improving cardiovascular tone, and inhibiting gastrointestinal, reproductive, and immune systems. The related cascade of hormones through the blood, known as the hypothalamic–pituitary–adrenal (HPA) axis, is activated by a wide variety of environmental and social stressors, in particular, exposure to novelty and lack of predictability of or control over important events (for reviews, see Sapolsky et al. 2000; Wingfield 2004). For example, chronic social competition or threat of competition can affect HPA functioning among animals with high (or low) social status (Virgin and Sapolsky 1997; Kotrschal et al. 1998; Goymann et al. 2001; Abbott et al. 2003; Creel 2005; Pride 2005). In addition, encounters with predators, their cues, or signals warning of them can elicit responses from adrenal glands, including secretion of epinephrine and glucocorticoids (e.g., Eilam et al. 1999; Hubbs et al. 2000; Cockrem and Silverin 2002; Rogovin et al. 2004; Apfelbach et al. 2005). In some species, glucocorticoid levels covary with life-history variables and predation risk, but the nature of these relationships depends on the season as well as animals' sex, age, and reproductive status (Fraser and Gilliam 1987; Boonstra and Singleton 1993; Boonstra and McColl 2000; Hik et al. 2001; Wingfield 2004; Reeder and Kramer 2005).

Belding's ground squirrels (*Spermophilus beldingi*) provide an ideal opportunity for studying the effects of predation risk on stress responses and behavior. *S. beldingi* predators, including coyotes (*Canis latrans*), badgers (*Taxidea taxus*), weasels (*Mustela* spp.), and various species of raptors (*Buteo*, *Accipiter*, *Falco*), evoke two types of alarm calls from adults. Trills are elicited by slow-moving predators such as coyotes and weasels that do not represent imminent danger, and whistles are elicited by fast-moving predators such as hawks that do present an immediate threat. Trills typically cause adults to post (a bipedal stance) and visually scan the area for what evoked the call, whereas whistles prompt animals to run to the nearest burrow, often entering the refuge (Sherman 1976; Mateo 1996; see also Owings and Virginia 1978; Schwagmeyer and Brown 1981; Hoogland 1995). Optimal responses to alarm calls (likelihood of responding, duration of alert behaviors, and types

of initial response) vary by age, habitat, and even specific locations within a site, and this plasticity is likely favored because predator environments change temporally and spatially. Moreover, juveniles (young of the year) learn from their mothers the best way to respond to alarm calls for their immediate area (Mateo 1996; Mateo and Holmes 1997, 1999). Predation can account for up to 60% of juvenile and 11% of adult mortality.

Time spent engaged in vigilance and antipredator responses reduces the amount of time available for other activities, most notably feeding. Overwinter survival in ground-squirrel species that are obligate hibernators depends on the accumulation of body fat before hibernation (Murie and Boag 1984). In *S. beldingi*, up to 70% of juveniles and 40% of adults do not survive hibernation (Sherman and Morton 1984; personal observation). Thus, for hibernating prey with limited active seasons in which to gain weight, there is an important trade-off between foraging and vigilance (see also Bachman 1993), favoring responses that are appropriate for local predator environments. Moreover, frequent increases in corticoids in response to predators or alarm calls can interfere with ongoing energy storage for the upcoming winter, which is mediated in part by lower corticoid levels (Sapolsky et al. 2000).

In this study, I explored the relationships among predation risk, behavior, and stress hormones in *S. beldingi* and whether they covary with features of local habitats. I studied three populations of adult *S. beldingi* that lived in different habitats and, thus, potentially experienced differences in predation pressure, which is defined here as the risk of being detected and caught by a predator and is influenced by the density, type, and visibility of predators as well as the conspicuousness of refugia, among other factors. Multiple phenotypic traits are affected by perceived predation risk; therefore, I collected behavioral data on activity budgets, frequency of alarm calls, and responses to playbacks of alarm calls and non-alarm calls. In addition, I collected fecal samples for noninvasive monitoring of glucocorticoid levels. Collectively, these measures were used to characterize the predation risk perceived by *S. beldingi*, as direct predator counts at these sites are unreliable. In addition, animals could face predation attempts with equal frequency at different sites, but if a prey animal's ability to detect oncoming predators or escape routes is compromised at one site, that animal may perceive a (and actually be at) greater risk. At each population, behavioral observations and fecal sampling began shortly after natal emergence of juveniles, when females had begun to cease lactating and males were gaining weight for hibernation. This period coincides with moderate levels of social interactions, particularly among females and juveniles. The study was conducted over 4 years because weather conditions, food availability, density of *S. beldingi*, and density and types of predators vary from year to year

(personal observation), and data from a single field season would likely not be representative of overall conditions in each of the areas.

Materials and methods

Animals

Belding's ground squirrels are diurnal social rodents that live in alpine and subalpine habitats throughout the Sierra Nevada and southern Cascade mountains. They are socially active above ground between April and August and hibernate the remainder of the year. Each mother produces one litter annually of five to eight pups, which is reared for a month in an underground burrow (the natal burrow); adult males do not provide paternal care. Young first come above ground (they 'emerge') as nearly weaned, 4-week-old juveniles (Sherman and Morton 1984; Mateo 1996). Females can live an average of 3.4 ± 0.3 years (up to 12 years); males live 2.1 ± 0.4 years (up to 9 years; Sherman and Morton 1984; personal observation).

Field research was conducted during the summers of 2002–2005 on three *S. beldingi* populations in the eastern Sierra of California within 100 km of Mammoth Lakes, CA: Mono County (Co.) Park, Lundy Canyon, and Rock Creek. These sites were chosen because they vary in habitat quality and, potentially, predation risk and because previous observations of *S. beldingi* suggested population differences in behaviors. However, I did not a priori characterize each site as being low, medium, or high in predation risk. I collected fecal samples from adults at the end of the reproductive season for 4 years and in 2 of those years (2003, 2004) continued to observe animals for approximately 2 weeks to document activity budgets and antipredator responses.

Mono Co. Park (elevation 1,966 m) is characterized by large, irrigated lawns bordered by willow bushes (*Salix* spp.) and a stream lined with willow trees and cottonwood trees (*Populus* spp.). Due to regular mowing at the park, the grass height was never higher than 5 cm. Predation attempts have been observed by bobcats (*Lynx rufus*), coyotes, dogs (*Canis lupus familiaris*), raptors (*Accipiter* spp.; *Buteo* spp.), and, on young juveniles, California gulls (*Larus californicus*) and California ground squirrels (*Spermophilus beecheyi*). The Lundy Canyon site (2,316 m) is a visually closed collection of small campsites surrounded tightly by aspen trees (*Populus tremuloides*) and tall grasses, with some mixed brush (*Artemisia*, *Purshia*, and *Chrysothamnus* spp.) along the north edge. Depending on the time of summer, the grasses and bushes (which were not managed) near burrow entrances ranged from 3 to ~70 cm and were typically >40 cm high at natal emergence of juveniles. Observed predators include coyotes, dogs, raptors, and *S.*

beecheyi. The Rock Creek Canyon site (specifically, Lower Horse Corral; 2,834 m) is a typical eastern Sierra subalpine meadow, bounded by Rock Creek, dry streambeds, and scattered willow bushes and aspen and pine trees (*Pinus* spp.). The main meadow's vegetation of grasses and wildflowers ranged naturally from 1 cm at both the beginning and the end of the active season to 15 cm high at the peak of wildflower growth. At this site, we have seen coyotes, dogs, martens (*Martes americana*), short- and long-tailed weasels (*Mustela erminea*, *Mustela frenata*), Northern goshawks, red-tailed hawks, and other raptors. Note that *S. beldingi* likely see many more predators than do human observers, and thus, our frequency of predator sightings underestimates actual predator types or densities. For example, at Lundy Canyon, more so than at the other sites, we do not see predators until long after *S. beldingi* detect them, and due to the density of the vegetation there, we have difficulty seeing each other let alone *S. beldingi* and their predators.

To characterize variation in behaviors and cortisol of free-living adults, I and my assistants collected behavioral data and fecal samples from *S. beldingi* at the three sites during the post-lactation period shortly after juveniles first emerged aboveground from natal burrows (only five fecal samples were collected at Lundy Canyon in 2005, and these were excluded from the analysis). Dates of emergence from hibernation in the spring are partially dictated by elevation, which affects the timing of mating. Therefore, the timing of juvenile emergence at the sites was staggered across the summer, allowing us to complete data collection on each population before moving to the next. At Rock Creek, adults were temporarily marked with hair dye (Lady Clairol blue-black; Clairol, Stamford, CT, USA) and permanently identified with numbered ear tags (one on each ear; National Band & Tag, Newport, KY, USA) or intrascapular passive integrated transponder tags (Biomark, Boise, ID, USA). Due to time constraints of other ongoing studies, adults at Mono Co. Park and Lundy Canyon were individually marked with ear tags in 2005 only. However, in all years at those sites, we clipped some hair from the dorsal region of all animals that were trapped, enabling us to avoid collecting fecal samples from animals that had been trapped recently. Therefore, the analyses did not include samples with artificially elevated corticoid levels due to the stress of recent trapping and handling, nor did it include more than one sample from an individual. After 2–3 days of fecal sampling, we collected time-budget data and conducted a playback study to document responses to alarm calls warning of predators for the next 10–14 days (in 2003 and 2004; see Table 1). During the study period, juveniles were still learning how to respond to auditory signals (Mateo 1996); therefore, their behavioral and hormonal data were omitted from these analyses (see Mateo 2006).

Table 1 Dates of playback studies, numbers of playbacks presented, and numbers of reactions recorded (individual squirrels were present during more than one playback)

Population	Dates	# of playbacks	# of reactions
2003			
Mono Co. Park	2–12 June	284	440
Lundy Canyon	17–28 June	151	165
Rock Creek	7–23 July	331	541
2004			
Mono Co. Park	1–11 June	316	492
Lundy Canyon	20–27 June	173	191
Rock Creek	2–12 July	250	338

Activity budgets

In 2003 and 2004, we collected data on the time budgets of adults in each of the populations. We used focal-animal sampling and instantaneous recording to document the behavior of an individual at 1-min intervals for 20-min periods (Altmann 1974). Focal samples were typically completed in the interval between playbacks, which were conducted approximately every 20 min (see below). A scan of a new individual was initiated when a focal adult was out of view for more than 5 min. Behaviors were divided into nine nonoverlapping categories (see Mateo 1995): (1) agonism, including lunging, chasing, and wrestling; (2) socializing (affiliative behaviors and nasal investigation); (3) stationary (laying prone or sitting on hindquarters, but not alert, chewing, or scanning the area); (4) feeding (chewing or manipulating a food item, including walking with nose to the ground ‘inspecting’ vegetation); (5) grooming, including scratching and dustbathing; (6) locomoting (walking or running, not including feeding bouts, social interactions, or antipredator behavior); (7) down burrow (entire body submerged belowground); (8) alert (any of the postures described under ‘behavioral response measures,’ below, while not feeding or resting); and (9) nest maintenance, including digging in soil and gathering nesting material.

I converted data from each focal scan to proportions (i.e., percentage of time spent engaged in each behavioral state during the 20-min scan) for all scans in which at least ten behavioral samples were recorded. Proportions of time spent in each behavior were not normally distributed, and transformations were unsuccessful; therefore, nonparametric analyses and post hoc tests were used (Siegel and Castellan 1988). Most *S. beldingi* were the target of focal samples on multiple occasions, to obtain a good representation of behaviors across the morning and across days. However, in an analysis of activity budgets restricted to known individuals at Rock Creek that participated in four or more samples ($N=42$ in 2003, $N=23$ in 2004), between-individual variation was not larger than within-individual

variation (unpublished data). Thus, the units of analysis for these data were the focal samples. The numbers of unique individuals observed are similar to the numbers of animals from which fecal samples were obtained (see “Fecal sampling”).

During these activity-budget scans, the frequency of *S. beldingi* alarm calls (whistles and trills) heard at each site was recorded. Calls were noted regardless of whether or not the caller was the focal animal. After years of working at these sites, I am confident that at least 90% of alarm calls can be heard from any location within the main observation areas. Calls judged to be elicited by humans, because they were made by a ground squirrel near a human and concurrent with the human’s approach or sudden movement, were not noted. Calls evoked by conspecifics, such as those intruding on a female’s territory, were also excluded. To ensure independence of the data, alarm-call data were recorded by one person. The number of calls heard was divided by the total number of hours of observation for each day, and data were analyzed for each year with Kruskal–Wallis one-way analyses of variance (ANOVA).

Quantifying antipredator responses

Auditory stimuli To examine differences in antipredator behavior at the three sites, we videotaped the responses of *S. beldingi* to playbacks of conspecific and heterospecific vocalizations in 2003 and 2004. We presented four categories of auditory stimuli: two types of *S. beldingi* alarm calls (whistles and trills), *S. beldingi* squeals (made by juveniles during rough play), and songs of house wrens, *Troglodytes aeodon*. I used squeals and wren songs as control stimuli to record responses to common auditory stimuli that are not associated with predator contexts. I used eight exemplars of each stimulus, selected for their signal amplitude and signal/noise ratio, and each exemplar within a category was recorded from a different individual. Digitized calls were played from a Sony TCM-5000 or TC-D5M tape player connected to a Mineroff SME-AFS speaker. All calls were presented at peak amplitudes approximating natural intensities. Alarm-call playbacks have been used successfully to elicit species-typical antipredator responses in *S. beldingi* (e.g., Mateo 1996) and additional details on the stimuli used in this study and how they were recorded and processed for playback are in Mateo (1996) and in Mateo and Holmes (1997).

Playback protocol We conducted playbacks daily between 0800 and 1300 hours. For each playback session, the four calls were presented in a balanced order approximately one playback every 20 min, unless we heard a natural alarm call or saw a potential predator during the preceding interval. Responses to playbacks were videotaped with Sony Digital8 HandyCam camcorders by observers sitting on

3-m elevated viewing platforms. We arbitrarily selected a focal ground squirrel and began filming when that individual was ≥ 3 m from a burrow. We filmed the focal ground squirrel from 15 s before stimulus onset until it resumed a non-alert behavior, such as feeding or socializing. Once all four call types were presented, an observer moved to another location within the site to record the responses of other individuals. *S. beldingi* continued to respond in a species-typical manner to natural and recorded alarm calls throughout the study, indicating that our playback schedule minimized habituation to the playbacks (see also Mateo 1996).

Behavioral response measures Pre- and post-playback behaviors were scored from videotapes using Ethos22 event-recorder software (G. Gerstner, University of Michigan). This program summarized the frequencies and durations (to the nearest 0.01 s) of six alert behaviors (horizontal, slouch, posting, and vertical-stretch postures, running and below ground) and four non-alert behaviors (stationary, feeding, grooming, and socializing; defined in Mateo 1995) before and after the playback stimulus. From the videotapes, I derived four response measures for each individual's response to a playback. (1) "Responsivity" indicates whether an individual displayed an antipredator response to a playback (change from a non-alert to one of the six alert behaviors, above). If an individual's behavior did not change after the playback, it was scored as not responding. If an individual responded to a stimulus, the following three measures were scored. (2) "Initial response" is the respondent's first observable reaction to a playback, categorized as entering a burrow, running to a burrow, posting, or freezing (or slightly raising its head without changing body position). (3) "Response duration" is the total time exhibiting any of the six alert behaviors, measured from the beginning of a response (typically concurrent with stimulus onset) until the individual resumed a non-alert behavior. Response durations were log-transformed for normality, and distributions were verified with Kolmogorov–Smirnov tests. (4) "Vigilant posture" quantified the maximum alert posture exhibited by an individual during any point of its response as follows: 4=below ground (full body enters a burrow for a minimum of 2 s), 3=post (standing on hindfeet with torso held straight, with or without legs fully extended), 2 =slouch (sitting on hindquarters, torso not fully extended), and 1=horizontal (head raised with three or four feet on the ground; see also Mateo 1996).

Tapes were scored by four people familiar with the study but unaware of any population-specific hypotheses. Alpha was set at 0.05, and data are presented as mean+SE. Because I was interested in population differences in responsiveness and levels of vigilance at the sites, rather than specific responses to each call type (e.g., Mateo 1996), I grouped the auditory stimuli into alarm calls (whistle and

trill) and non-alarm calls (squeal and wren song). We often videotaped the responses of more than one individual to a playback, but variation in responses within a playback was as great or greater than variation in responses to all playbacks of that call type (unpublished data); therefore, all responses to one playback were included in analyses. Most animals were nursing females still faithful to the area around their natal burrow, and thus, although animals were not individually identifiable at Mono Co. Park or Lundy Canyon, we could generally keep track of particular individuals. We also switched focal animals frequently within a day and systematically moved our locations within and across days. Thus, most animals at each site were represented in both the activity-budget and playback datasets.

Fecal sampling

I used a noninvasive fecal measure of cortisol, validated specifically for Belding's ground squirrels, that provides an integrated measure of circulating cortisol in *S. beldingi* over the previous 12–24 h (Mateo and Cavigelli 2005). This method is preferable to the use of serum samples that would reflect acute responses related to the immediate stress of trapping and handling. In addition, fecal metabolites reflect unbound active portions of glucocorticoids and are relatively unaffected by real or perceived stressors (Palme et al. 2005). Efforts were made to sample from all animals at each site, but because males are socially and spatially peripheralized after mating, their sample sizes were smaller than those of females. Each collection period lasted 2–3 days and was limited to 0700–1200 hours to minimize circadian variation in fecal metabolites (Cavigelli et al. 2005; Mateo, unpublished data). I took advantage of the fact that ground squirrels typically defecate while in traps (single-door live traps; Tomahawk, Tomahawk, WI, USA). Therefore, immediately after an animal entered a trap, the trap was moved aside, and the fecal pellets, in the vegetation which was under the trap, were collected with clean tweezers and placed in polypropylene microcentrifuge tubes (Cole Parmer, Vernon Hills, IL, USA). If animals did not immediately defecate, their trap was set inside a clean plastic bucket until defecation. Tweezers, traps, and buckets were cleaned with Cide-All® germicidal detergent (Chemifax, Santa Fe Springs, CA, USA). Feces contaminated with urine (visibly wet and/or in a pool of urine) were discarded; see Cavigelli et al. 2005 for more on how urine can alter fecal corticoid levels. Animals were sexed and weighed after fecal collection and marked (if needed) as described above. Samples were stored immediately in a cooler with ice packs until placement in a freezer at a nearby field station at -15°C and were then transferred to a -80°C freezer at the end of the field season (4–6 weeks later). At Mono Co. Park, I sampled 5 males and 19 females in 2002, 1 and 18 in

2003, 8 and 24 in 2004, and 16 and 16 in 2005. At Lundy Canyon, I sampled 2 males and 11 females in 2002, 2 and 9 in 2003, and 6 and 16 in 2004. At Rock Creek, I sampled 8 males and 30 females in 2002, 20 and 32 in 2003, 7 and 16 in 2004, and 12 and 18 in 2005.

Fecal cortisol metabolite assays

Fecal corticoids were extracted following the methods outlined in Mateo and Cavigelli (2005). Samples were assayed with cortisol solid phase component system ^{125}I -cortisol Corticote[®] radioimmunoassay kits (MP Biomedicals, Irvine, CA, USA) and were assayed in duplicate and reanalyzed if the coefficient of variation between duplicates exceeded 20%. The sensitivity of the assay is 0.07 $\mu\text{g}/\text{dl}$, according to the manufacturer. Two control samples, each made by pooling fecal extracts from five animals, were analyzed in every assay (the 'low' pool, approximately 60–70% binding and the 'high' pool, approximately 20–30% binding). Based on repeated analyses ($n=50$ assays using nine different batches of each pool) of the low and high pools, mean intra-assay coefficients of variation for the assays were 9.08% for the low pool and 6.19% for the high pool. Mean inter-assay coefficients of variation were 11.12% for the low pool and 10.90% for the high pool. Fecal corticoid levels were divided by the proportion of isotope recoveries and are expressed as nanogram per gram of dried feces. Cortisol metabolite data were log-transformed for normality, and distributions were verified with Kolmogorov–Smirnov tests. Extracted samples were assayed after varying periods in deep storage (-80°C ; 0.5 to 3.5 years); it is unknown whether *S. beldingi* metabolites degrade during long-term storage (e.g., Khan et al. 2002; Beehner and Whitten 2004); therefore, I did not make statistical comparisons between study years.

Results

Activity budgets across three populations

Of the nine behavioral categories, only a few differed across populations. In 2003, adults at Mono Co. Park and Lundy Canyon spent significantly more of their time budgets locomoting than adults at Rock Creek ($KW_2=6.952$, $p=0.031$; Fig. 1a). Adults at Lundy Canyon spent more time alert and less time feeding than adults at the other two sites ($KW_2=19.20$, $p<0.0001$; $KW_2=15.04$, $p=0.001$, respectively).

In 2004, *S. beldingi* at Mono Co. Park spent a greater proportion of their active time locomoting than adults at Lundy Canyon ($KW_2=6.619$, $p=0.037$; Fig. 1b). Adults at Lundy Canyon and Mono Co. Park also spent significantly more time alert than those at Rock Creek ($KW_2=12.693$,

$p=0.002$), and Lundy adults spent significantly less time feeding than adults at the other sites ($KW_2=17.984$, $p<0.0001$).

There were significant differences in the rate at which *S. beldingi* emit alarm calls at the three sites. In 2003, calls were heard significantly less often at Lundy Canyon than at Rock Creek (mean number of calls per hour \pm SE: Mono Co. Park, 2.82 ± 1.17 ; Lundy Canyon, 1.33 ± 0.18 ; Rock Creek, 2.72 ± 0.34 ; overall $KW_2=8.37$, $p=0.015$; Bonferroni corrected $\alpha=0.0167$, post hoc comparison $p=0.006$). In 2004, calls were heard significantly less often at Lundy Canyon than at Mono Co. Park (Mono Co. Park, 3.78 ± 0.65 ; Lundy Canyon, 1.25 ± 0.23 ; Rock Creek, 2.22 ± 0.48 ; overall $KW_2=11.18$, $p=0.004$; Bonferroni corrected $\alpha=0.0167$, pairwise post hoc comparison $p=0.001$).

We attempted to record a behavior every minute, yet sometimes animals were out of view. Descriptively, animals were visible to observers less often at Lundy Canyon than at the other two sites. I divided the total number of behavioral states recorded at each site by the total number of minutes of observation. In 2003, a behavior was recorded during a focal-animal sample approximately every 2.42 minutes at Lundy Canyon compared with every 1.88 minutes at Mono Co. Park and 1.87 minutes at Rock

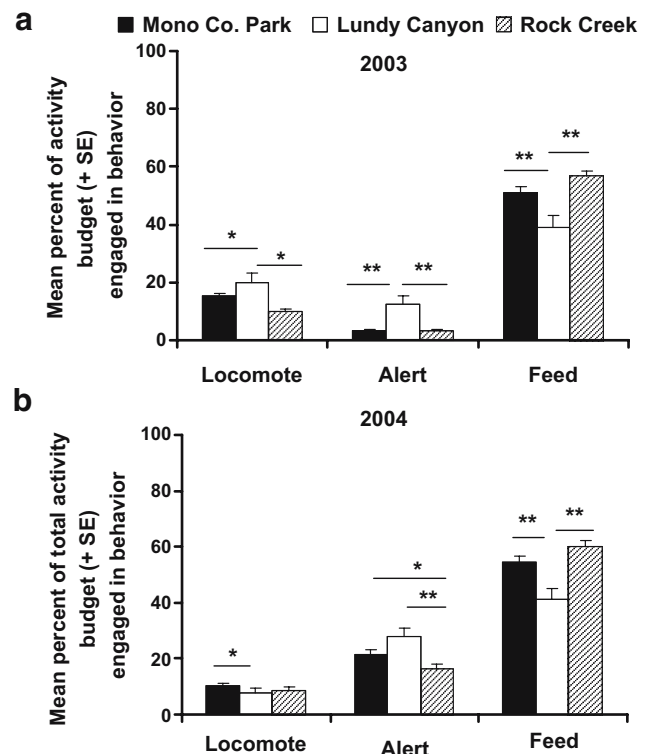


Fig. 1 Population differences in mean (+SE) proportion of time adult *S. beldingi* engaged in various behaviors. **a** Data from adult *S. beldingi* in 2003. **b** Data from adult *S. beldingi* in 2004. Only behaviors that differed significantly across populations are shown. Lines over columns indicate pairwise post hoc population differences, significant after Bonferroni adjustments at the 5% (single asterisk) and 1% (double asterisks) levels. See text for statistical details

Creek. In 2004, it took 2.71, 1.92, and 1.60 minutes at the three sites, respectively.

Antipredator responses of three populations

In 2003, adults in each population were equally likely to respond to playbacks of alarm calls ($\chi^2(2)=2.30$, $p=0.32$), but those at Mono Co. Park were less responsive to playbacks of non-alarm calls than adults at the other sites ($\chi^2(2)=10.91$, $p=0.004$; Fig. 2a). Among the animals that exhibited a response to calls, there were population differences in the length of time responders spent alert (alarm calls: $F_{2,502}=33.98$, $p<0.0001$; non-alarm calls: $F_{2,181}=5.97$, $p=0.003$), with Lundy Canyon *S. beldingi* remaining alert significantly longer than adults at the other sites in response to both call types (post hoc pairwise *t*-test comparisons with Bonferroni corrected $\alpha=0.0167$: Lundy Canyon vs Mono Co. Park $t_{225}=8.60$, $p<0.0001$, Lundy Canyon vs Rock Creek $t_{365}=3.42$, $p=0.0007$ for alarm calls; Lundy Canyon vs Mono Co. Park $t_{81}=3.60$, $p=0.0005$, Lundy Canyon vs Rock Creek $t_{131}=2.60$, $p=0.01$ for non-alarm calls), and Rock Creek adults responding to alarm calls longer than adults at Mono Co. Park ($t_{414}=6.12$, $p<0.0001$; Fig. 3a). Initial responses to alarm calls varied

by population as well (overall $\chi^2(6)=26.44$, $p<0.0001$; Fig. 4a), and partitioning of the tables revealed that adults at Lundy Canyon were significantly more likely to run to a burrow in response to a call than adults at Rock Creek ($p=0.0003$). There were no population differences in initial responses to non-alarm calls (overall $\chi^2(6)=10.12$, $p>0.10$). Finally, the three populations expressed different levels of vigilance in their total responses to alarm call playbacks (overall $KW_2=40.84$, $p<0.0001$), with adults at Lundy Canyon showing more exaggerated responses than adults at the other sites and Rock Creek adults exhibiting more vigilant responses than those at Mono Co. Park (all post hoc $ps<0.0001$). In response to non-alarm calls, adults at Mono Co. Park showed less exaggerated responses than those at Lundy Canyon and Rock Creek (overall $KW_2=7.07$, $p=0.029$; post hoc comparisons $p=0.04$ and $p=0.05$, respectively; Fig. 5a).

In 2004, there were no significant population differences in the likelihood of responding to playbacks of alarm calls or non-alarm calls ($\chi^2(2)=3.66$, $p=0.16$ and $\chi^2(2)=1.81$, $p=0.40$, respectively; Fig. 2b). When adults responded to

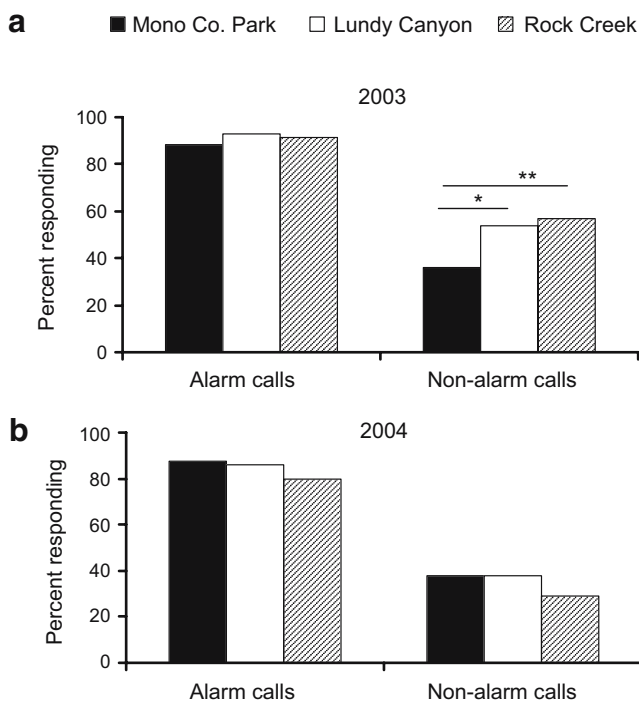


Fig. 2 Percentage of adults from three populations responding to playbacks of alarm calls (*S. beldingi* whistles and trills) and non-alarm calls (*S. beldingi* squeals and house-wren songs). **a** Data from adult *S. beldingi* in 2003. **b** Data from adult *S. beldingi* in 2004. Lines over columns indicate significant population differences ($*p<0.05$; $**p<0.01$). See text for statistical details

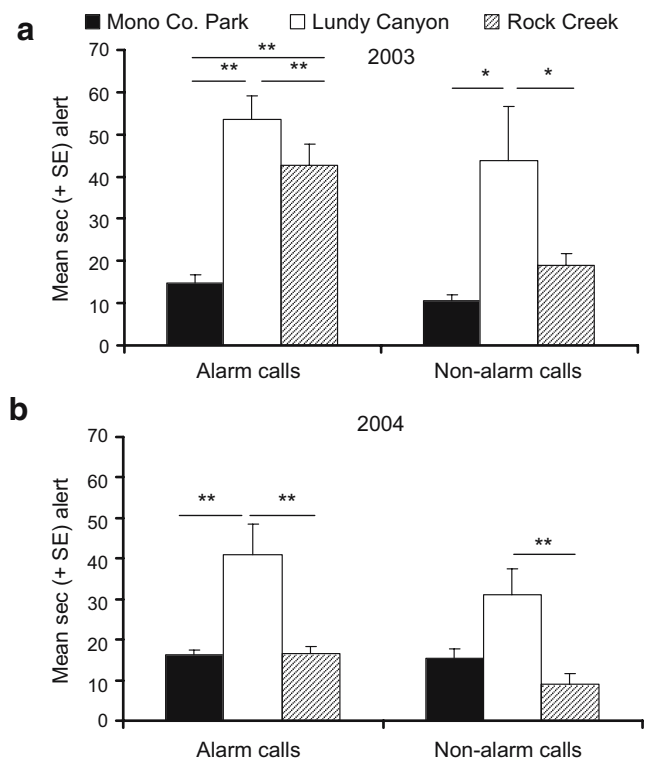


Fig. 3 Response durations (mean $s\pm SE$) of adults that responded to playbacks, measured from presentation of the playback stimulus until non-alert behavior was resumed. **a** Data from adult *S. beldingi* in 2003. **b** Data from adult *S. beldingi* in 2004. Lines over columns indicate population differences based on post hoc pairwise *t*-test comparisons following significant overall ANOVA on log-transformed data, significant after Bonferroni adjustments at the 5% (single asterisk) and 1% (double asterisks) levels. See text for statistical details

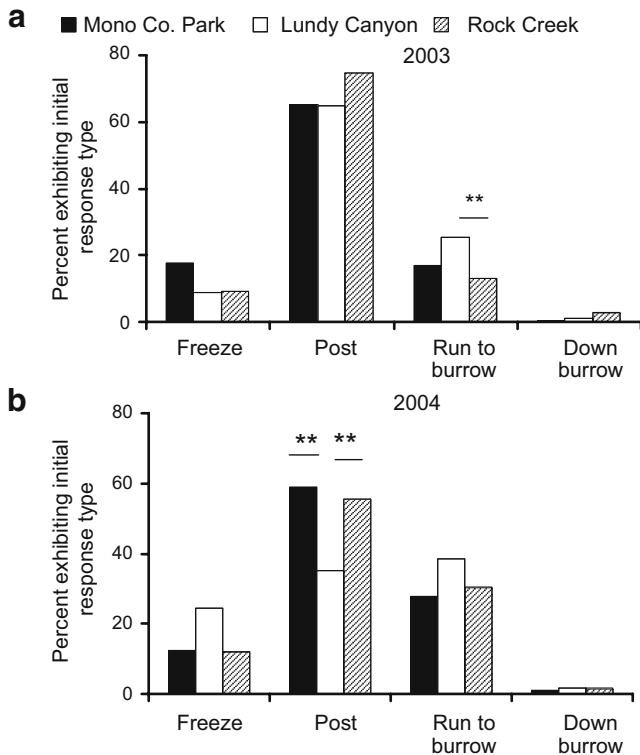


Fig. 4 Percentage of adults responding to alarm-call playbacks with each of four initial response types. **a** Data from adult *S. beldingi* in 2003. **b** Data from adult *S. beldingi* in 2004. Lines over columns indicate significant population differences based on partitioned tables (** $p < 0.01$). See text for statistical details

playbacks, those at Lundy Canyon remained alert longer than adults at the other sites (alarm calls: $F_{2,362} = 5.41$, $p = 0.005$; non-alarm calls: $F_{2,97} = 7.99$, $p = 0.001$; post hoc pairwise t -test comparisons with Bonferroni corrected $\alpha = 0.0167$: Lundy Canyon vs Mono Co. Park $t_{243} = 2.67$, $p = 0.008$, Lundy Canyon vs Rock Creek $t_{209} = 2.88$, $p = 0.004$ for alarm calls; Lundy Canyon vs Rock Creek $t_{50} = 3.94$, $p = 0.0003$ for non-alarm calls; Fig. 3b). Initial responses to alarm call playbacks varied by population (overall $\chi^2(6) = 23.53$, $p = 0.001$; non-alarm calls $\chi^2(6) = 11.37$, $p = 0.077$), and partitioning of the tables revealed that adults at Lundy Canyon were significantly less likely to post in response to an alarm call than adults at Mono Co. Park and Rock Creek ($p < 0.0001$ and $p = 0.006$, respectively; Fig. 4b). Lundy adults also showed more vigilant initial responses to alarm calls and non-alarm calls (KW₂ = 6.02, $p = 0.049$; KW₂ = 8.454, $p = 0.015$, respectively; all post hoc comparison $ps < 0.001$; see Fig. 5b).

At Rock Creek, where adults were individually marked and thus sex and age were known, there were no significant differences between males and females (2003, 21 males, 34 females; 2004, 10 males and 16 females) or between adults (>2 years of age; $N = 46$ in 2003; $N = 20$ in 2004) and yearlings ($N = 9$ in 2003; $N = 6$ in 2004) in response

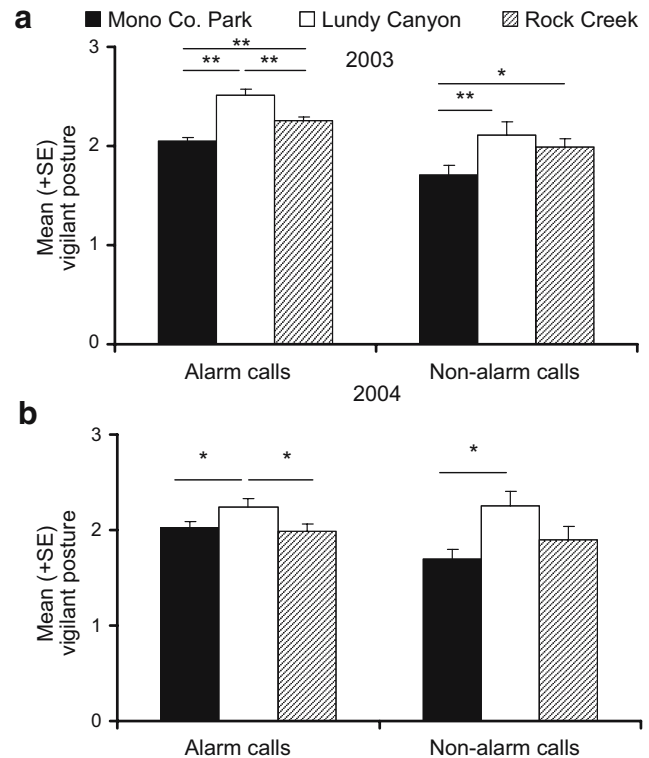


Fig. 5 Vigilant-posture scores (mean+SE) of adults responding to playbacks of alarm calls and non-alarm calls. **a** Data from adult *S. beldingi* in 2003. **b** Data from adult *S. beldingi* in 2004. Vigilant-posture scores, which are ordinal data (ranked 1–4), are presented as means for illustrative purposes. Lines over columns indicate significant population differences (* $p < 0.05$; ** $p < 0.01$). See text for statistical details

likelihood, initial responses, duration alert, or vigilant postures, using χ^2 tests, one-way ANOVA, and Kruskal–Wallis ANOVA (all $ps > 0.10$ s). Anecdotally, animals were more visible at Mono Co. Park and Rock Creek than at Lundy Canyon. In 2003, we recorded the responses of 1.55, 1.63, and 1.09 animals for every playback at each of the sites, respectively. The pattern was similar in 2004, with 1.55, 1.35, and 1.10 animals observed for each playback, respectively.

Fecal corticoid levels

In each year of the 4-year study, there were significant differences in fecal corticoids across populations (Fig. 6; 2002: overall $F_{2,72} = 10.92$, $p < 0.0001$; Mono Co. Park vs Rock Creek: $t_{60} = 4.22$, $p < 0.0001$; Lundy Canyon vs Rock Creek: $t_{49} = 2.67$, $p = 0.01$; 2003: overall $F_{2,79} = 4.56$, $p = 0.013$, Mono Co. Park vs Lundy Canyon: $t_{28} = 3.86$, $p = 0.001$; Mono Co. Park vs Rock Creek: $t_{69} = 2.74$, $p = 0.008$; 2004: overall $F_{2,78} = 6.124$, $p = 0.003$, Mono Co. Park vs Lundy Canyon: $t_{56} = 3.01$, $p = 0.004$; Lundy Canyon vs Rock Creek: $t_{47} = 3.95$, $p < 0.0001$; 2005: $F_{1,59} = 93.81$, $p < 0.001$). Levels of cortisol

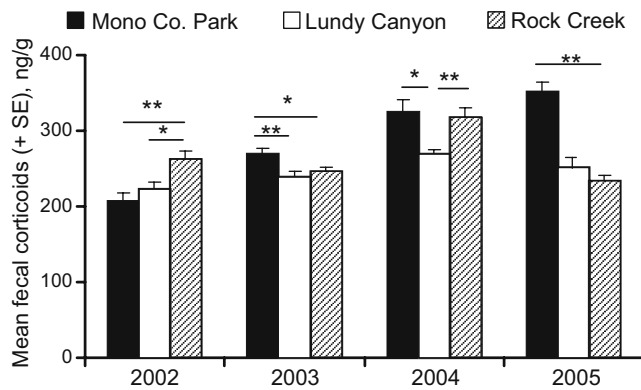


Fig. 6 Patterns of geographic variation in fecal corticoids (ng/g dried feces+SE) of free-living adult *S. beldingi* sampled during the period of juvenile emergence from natal burrows. Lines over columns indicate population differences based on post hoc pairwise *t*-test comparisons following significant overall ANOVA on log-transformed data, significant after Bonferroni adjustments at the 5% (single asterisk) and 1% (double asterisks) levels. Data for Lundy Canyon in 2005 are shown for illustrative purposes only. See text for statistical details

metabolites in Lundy Canyon animals were always lower than those at Rock Creek or Mono Co. Park or both. There were no significant sex differences in cortisol metabolite levels within populations in any year, with the sole exception of Mono Co. Park in 2002, when males had significantly higher levels than females, due in part to one male with an exceptionally high level (unadjusted mean±SE, females 199.68±8.86 ng/g, males 303.21±67.68 ng/g; $F_{1,23}=6.52$, $p=0.018$). Nor were there significant differences at any site between samples known to come from yearlings and adults (>2 years of age; Mono Co. Park: $N=20$ and $N=81$, respectively; Lundy Canyon: $N=15$ and $N=35$, respectively; Rock Creek: $N=29$ and $N=111$, respectively; ANOVA, $p_s>0.10$) or between females known to have litters emerge and those who did not (Mono Co. Park: $N=41$ and $N=13$, respectively; Lundy Canyon: $N=27$ and $N=1$, respectively (analysis not conducted); Rock Creek: $N=69$ and $N=11$, respectively; one-way ANOVA, $p_s>0.10$).

Discussion

Three converging lines of evidence indicate that Belding's ground squirrels living in Lundy Canyon perceived a greater predation risk than those living at Mono Co. Park or Rock Creek. First, adults at Lundy Canyon spent more time alert and less time foraging than *S. beldingi* at the other sites (Fig. 1). Second, although adults at all sites were equally likely to react to alarm calls (*S. beldingi* whistles and trills) and non-alarm calls (*S. beldingi* squeals and house-wren songs; Fig. 2), those in Lundy Canyon remained alert almost twice as long as adults at the other sites in response to both call types (Fig. 3). Third, Lundy

Canyon animals tended to show more exaggerated initial reactions to calls and were more vigilant in their overall responses than adults at Rock Creek or Mono Co. Park (Figs. 4, 5). Together, these data indicate that *S. beldingi* living in Lundy Canyon perceive a greater predation threat when auditory signals are heard, particularly those warning of predators and throughout the day behave in ways that maximize vigilance and the chances of successful escapes.

These population differences in vigilant and antipredator behaviors were reflected in the hormone profiles of adults. Fecal corticoid metabolites represent circulating levels of cortisol during the previous 12–24 h (Mateo and Cavigelli 2005), including changes elicited by stressors from conspecifics and the predator cues to which an animal has been exposed. Stress-induced functions of glucocorticoids include increasing available glucose, improving cardiovascular tone, and inhibiting gastrointestinal, reproductive, and immune systems. However, this elevation of glucocorticoids in response to stressors can come at the cost of energy storage, particularly during periods of physical development or preparation for hibernation or migration (reviewed in Sapolsky et al. 2000; Reeder and Kramer 2005). Here, corticoid metabolites of *S. beldingi* were excreted at significantly lower levels by adults at Lundy Canyon than animals at Mono Co. Park and Rock Creek (Fig. 6). There was year-to-year variation in relative levels of corticoids from the three populations, which is not surprising given that each animal was only sampled once and those values can vary not only across the day but also across years. Collectively, the hormonal and behavioral data show that *S. beldingi* at Lundy Canyon are more vigilant and exhibit low average cortisol-metabolite levels compared with adults at the other sites. When reacting to predators or alarm calls, these lower corticoids allow Lundy Canyon animals to mount large acute responses (Mateo, unpublished data) and mobilize glucose for rapid and exaggerated escape movements and prolonged antipredator responses (see also Astheimer et al. 1995; Casolini et al. 1997; Virgin and Sapolsky 1997; Blanchard et al. 1998; Romero 2002; Boonstra 2005; Brown et al. 2005). These elevated corticoids, present in blood within a few minutes of a stressor, can then facilitate responses during a predator encounter, particularly if the predator continues to hunt in that area or if *S. beldingi* are unsure of its location, or they can affect responses to subsequent predator attacks (see 'stimulating' and 'preparative' actions in Sapolsky et al. 2000). Importantly, low corticoids might also facilitate glucose storage to offset the costs of the relatively decreased foraging efforts at Lundy Canyon (Sapolsky et al. 2000), as body weight accumulated in late summer is critical for overwinter survival as well as reproductive condition the following spring (Morton 1975; Bachman 1993; personal observation). The data presented here suggest that, in *S. beldingi*,

the competing demands of glucose storage and mobilization are balanced differently depending on the animals' local environments (see also Cavigelli 1999), and future studies could quantify glucose concentrations across sites throughout the active season (*sensu* Boonstra and McColl 2000).

Although natural selection may favor the same behavioral repertoire in all members of a species, temporal and spatial changes in local environments can lead to quantitative or qualitative differences among animals living in different populations (e.g., Endler 1995; Foster 1999). For example, at the twelve locations where I have observed them, *S. beldingi* produce both trill and whistle alarm calls and listeners respond in species-typical manners to the two call types. Yet, antipredator repertoires are plastic, with response likelihood, initial reactions, and duration of vigilance varying across contexts, allowing adult and juvenile animals to exhibit responses which are appropriate for their local predator environment (e.g., Mateo 1995, 1996; Mateo and Holmes 1999; see also Loughry 1988; Owings et al. 2001). Likely due to the tradeoff between foraging and vigilance (data presented here; see also Sherman 1976; Bachman 1993; Clinchy et al. 2004), adult *S. beldingi* living in sites with high predation risk such as Lundy Canyon spend more time alert and less time foraging than those in areas with less predation risk.

In addition, *S. beldingi* stress hormones are inversely related to perceived predation risk, with lower cortisol metabolite levels associated with higher predation risk and vigilance. This pattern is opposite to what might be expected when animals frequently experience stressors. For example, snowshoe hares (*Lepus americanus*) experiencing high predation rates exhibit larger acute cortisol responses than those during lower predation periods or when supplemented with food (Boonstra et al. 1998). Similarly, Arctic ground squirrels (*Spermophilus parryii*) living in forest habitats have lower acute responses compared with those living in boreal habitats, where vegetation is dense, and predators are observed more often (Hik et al. 2001). In contrast, European blackbirds (*Turdus merula*) living in urban environments, and presumably experiencing frequent anthropogenic stressors, have lower acute responses than birds living in forest habitats (Partecke et al. 2006). Further, young American kestrels (*Falco sparverius*) do not show acute corticosterone responses to playbacks of calls warning of predators (Duffy and Crandall 2005). Studies focusing on the relationships between basal glucocorticoids and predation have yielded mixed results. Corticosterone metabolites are positively associated with predator sightings in juvenile but not adult great gerbils (*Rhombomys opimus*; Rogovin et al. 2004). In several species, exposure to predators or their models or to vocalizations warning of predators does not reliably predict glucocorticoid levels (Cavigelli 1999; Cockrem and Silverin 2002; Pride 2005; Ylönen et al.

2006). These disparate findings reflect not only research on acute rather than basal glucocorticoids but can also reflect variation in predictability of encounters with predators. That is, *S. beldingi* living in Lundy Canyon might always perceive high predation risk and, because risk is predictable, exhibit low fecal corticoid levels (perhaps mediated proximately by down-regulation of glucocorticoid receptors in the brain; Sapolsky et al. 2000). Predation risk is relatively lower but less predictable for animals living in Mono Co. Park and Rock Creek, which might account for their higher cortisol metabolites (and higher rates of alarm calling). Thus, similar to the variable effects of social status on glucocorticoids, with higher levels sometimes associated with dominance and sometimes with subordination (e.g., Virgin and Sapolsky 1997; Kotrschal et al. 1998; Goymann et al. 2001; Abbott et al. 2003; Creel 2005; Pride 2005), corticoids may not show a one-to-one relationship with predation risk within or across species. Accordingly, the use of a multidimensional approach, including behaviors such as time budgets, space use, vigilance displays, alarm calls, and responses to cues of predators, rather than a single measure of responsiveness, is recommended to best characterize associations between adrenal hormones and predator contexts.

Any number of ecological parameters could contribute to these population differences, such as nutritional, climatic or social stressors, elevation, habitat quality (e.g., availability of refugia, visibility of approaching predators), density of ground squirrels, or density and types of predators. At Lundy Canyon, visibility is poor due to dense vegetation, and detection of predator and escape burrows is more difficult than at the other sites (personal observation). Antipredator behaviors can vary within species as a function of geographic location (e.g., Herzog and Schwartz 1990; Downes and Adams 2001) and habitat type (e.g., Metcalfe 1984; LaGory 1986; Goldsmith 1990; Alberts 1994; Schooley et al. 1996; Sharpe and Van Horne 1998; Blumstein et al. 2004). These differences might result from direct experience in the environments (such as hearing alarm calls, observing conspecific responses, or detecting a predator), from variation in physiological functioning that modulates behaviors in location-specific ways, or from adaptive radiation or genetic drift (Foster 1999). Finally, population differences in basal cortisol might reflect differences in HPA regulation (perhaps set early in life; Casolini et al. 1997; McCormick 1998; Hayward and Wingfield 2004; Bian et al. 2005; Mateo, unpublished data), rather than ongoing differences in 'psychological' stress due to recent experiences.

The phenotypic differences observed among the three populations of *S. beldingi* could be interpreted differently than I have done here. That is, the data might suggest that adults at Lundy Canyon perceive less predation risk than those at the other sites and, therefore give fewer alarm calls,

can afford to remain longer when responding to calls and produce lower levels of fecal corticoids. It is difficult to know what exactly the animals are perceiving, and to date, we know too little about predation and glucocorticoids in free-living animals to predict their relationships a priori (see above). However, this interpretation is not logical when considering the broader context of *S. beldingi* annual cycles and the significantly lower rates of foraging by Lundy Canyon animals. If animals there did experience relatively little predation risk, I would expect them to maximize their food intake and accumulation of body fat for the upcoming hibernation. Total body lipids increase about 15-fold during the last month of the active season (Morton 1975) and are critical for overwinter survival of sciurids (Murie and Boag 1984; Bachman 1993; Boswell et al. 1994). Among *S. beldingi*, up to 70% of juveniles and 40% of adults do not survive hibernation in a given year (Sherman and Morton 1984; personal observation). Indeed, during each of the 4 years of this study, adults at Lundy Canyon had lower body weights (taken during fecal collection) than adults at the other sites, significantly so in 2 years. In addition, the lower rate of alarm calling at Lundy Canyon compared with the other sites could reflect the difficulty of detecting predators due to the dense vegetation. Thus, given the competing needs of weight gain and vigilance, the behavior of Lundy Canyon animals can be most parsimoniously attributed to heightened perceived predation risk, relative to those at the other sites.

In this study, I did not quantify the numbers of predators that actually appeared or the frequencies of predation attempts at each site, due to the obvious potential bias in sampling among habitats that varied in visual openness. However, vigilant behaviors and stress hormones are influenced by both potential and actual threats in the environment and, thus, reflect perceived predation risk. For example, Mono Co. Park and Rock Creek are visually open sites, where predators and escape burrows can be seen by ground squirrels from a distance. In addition, *S. beldingi* at these sites can typically see who is giving a trill alarm call and in what direction the caller is looking, which helps listeners to locate the predator eliciting the call. In contrast, Lundy Canyon is a visually closed habitat where trees and tall grasses inhibit visual detection of predators and hide burrow entrances. The vegetation also obstructs transmission of auditory cues, making it difficult to localize alarm calls, particularly pure-tone whistles, and thus the predators that elicited them (Richards and Wiley 1980). The restricted visibility at this site might explain why animals were so frequently out of view during activity-budget scans and why alarm calls were heard infrequently, compared with animals at Mono Co. Park and Rock Creek. These population differences are not necessarily uniform, however, because within a population, antipredator behaviors and

cortisol levels can vary as a function of location. For example, adult and juvenile *S. beldingi* whose primary burrows and activity centers are located at the edge of the meadow at Rock Creek are more responsive to alarm calls and remain alert longer than those in the center, and those on the edge also have significantly lower cortisol metabolites than those that occupy the center of the meadow (presumably a safer location; Elgar 1989; Mateo 1996; unpublished data). Thus, in addition to species-specific and population-specific antipredator strategies, microhabitats and individual experiences will contribute to variation in strategies within a species.

Predators can exert direct selection pressures, by causing serious injury or death, but indirect predation pressures via vigilance, foraging, and locomotor demands can also have profound effects on prey anatomy, physiology, and behavior. Belding's ground squirrels exhibit stable population differences in patterns of foraging, vigilance, and antipredator behaviors, which correspond to features of their microhabitats. In particular, animals that appear to perceive greater predation risk exhibit lower fecal corticoid levels, which suggests that risk is predictable and thus animals do not experience chronic stress, and might lead to a more responsive HPA axis when responding to stressors. Glucocorticoids are involved in almost all aspects of an individual's life, including growth and maintenance, daily and seasonal rhythms, cognitive functioning, and responses to stressors. Therefore, selection might favor the evolution of stress-hormone levels that correspond to local predation pressure. That is, glucocorticoids will reflect environmental stressors in different populations or at different times of year and will covary with the expression of antipredator strategies such as shifts in microhabitat use, activity budgets, vigilance, and responses to predators and their cues. Future studies could examine the extent to which geographic differences in HPA functioning and survival behaviors are the result of direct experiences in those environments, maternal effects, and/or genetic variation. Finally, because these data are correlational, future empirical work can investigate the causal mechanisms associated with population differences in antipredator behaviors and glucocorticoids.

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