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Developmental and geographic variation in stress hormones in wild Belding's ground squirrels (*Spermophilus beldingi*)

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Abstract

Extensive research has been conducted on the role of glucocorticoids in regulating growth, mobilizing energy, responding to stressors and modulating learning and memory. However, little is known about the production of corticoids during early development in free-living animals, particularly during sensitive periods of acquisition of important behaviors. In a four-year study of Belding's ground squirrels, *Spermophilus beldingi*, a non-invasive assay of glucocorticoids was used to quantify age and population differences among juveniles from three California locations. Fecal–cortisol metabolites are elevated during a short period when juveniles first emerge aboveground from their natal burrows at about 4 weeks of age. This period of cortisol elevation coincides with when young are learning survival behaviors such as anti-predator responses and foraging strategies. Population differences in juvenile cortisol levels, which may reflect local variation in habitat quality and predator environments, were not evident until 2 weeks after emergence. Elevated cortisol at the age of emergence was also observed in juveniles born and reared in captivity without exposure to typical stressors that occur around the age of emergence. These results indicate that corticoids are regulated during early development, and the possible functions of age-related corticoid levels are discussed, including mobilization of glucose for natal emergence and later facilitation of growth and energy storage during the short summer before hibernation. In some species, elevated corticoids can also facilitate learning and memory, and current work is exploring whether the higher cortisol observed in all three *S. beldingi* populations just after emergence function to promote rapid acquisition of survival behaviors.

Keywords: *Spermophilus*; Ground squirrels; Cortisol; Stress; Fecal glucocorticoids; Development; Population differences

Introduction

Adrenal hormones regulate many facets of homeostasis, including responses to unpredictable environmental perturbations. Adrenal glucocorticoids regulate energy mobilization and storage and circadian rhythms, and they protect the body during and after the stress response, defined as the cumulative physiological reactions triggered by unpredictable events. 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, 1992; Apanius, 1998; Wingfield et al., 1998; Koolhaas et al., 1999).

The field of behavioral ecology is increasingly integrating proximate and ultimate levels of analysis, including investigations of how stressors faced by free-living animals affect physiology and reproductive success. Acute stress responses are triggered by unpredictable events such as predator attacks, sudden severe weather or agonistic social interactions, whereas chronic stress stems from long-term adverse environmental conditions, such as unpredictable decreased food supply or either low or high social status (e.g. Morton and Sherman, 1978; Kotrschal et al., 1998; Lima, 1998; Cavigelli, 1999; Hubbs et al., 2000; Creel, 2001; Goymann et al., 2001; Abbott et al.,

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2003; Sands and Creel, 2004). Both types of stress can directly influence survival and reproduction (reviewed in Buchanan, 2000; Wingfield, 2004; see also Jennings et al., 2004; Boonstra, 2005; Spencer et al., 2005), yet surprisingly very little is known about the activity of glucocorticoids and the HPA axis in early development.

Glucocorticoids can modulate the acquisition and retrieval of new memories (reviewed in McEwen and Sapolsky, 1995; Lupien and McEwen, 1997; McGaugh, 2000). Despite extensive research on the effects of glucocorticoids on cognition in humans and laboratory rodents, we know little about how these hormones influence biologically relevant learning in natural contexts. Belding's ground squirrels (*Spermophilus beldingi*) provide an ideal opportunity for studying the effects of stress on learning during early development, because much is known about the sources of stress young animals can encounter. *S. beldingi* are preyed upon by aerial and terrestrial predators which are signaled by whistle and trill alarm calls, respectively, each of which elicits a specific evasive response by listeners (Sherman, 1985; Mateo, 1996). Juvenile *S. beldingi* learn to respond appropriately to these two alarm calls during the first 5 days after initial emergence from natal burrows. This rapid learning of appropriate responses is important because up to 30% of juveniles disappear during the first 2 weeks after emerging aboveground at about 1-month of age, presumably due to predation (Mateo, 1996). Predation can account for up to 60% of juvenile *S. beldingi* mortality (Sherman and Morton, 1984; personal observation) and overwinter survival in some ground squirrels depends on the accumulation of body fat prior to hibernation (Murie and Boag, 1984), thus creating a trade-off between foraging and vigilance (see also Bachman, 1993). In addition to threats from predators, newly emergent juveniles are vulnerable to infanticide by adults and are in the process of becoming nutritionally independent (Sherman, 1982; Mateo, 1996).

Learning of appropriate anti-predator behaviors thus occurs during a time of considerable potential stress from predators, infanticidal conspecifics, late-season snowstorms and declining food availability. Here I report on developmental patterns of cortisol secretion in free-living juveniles which might facilitate or inhibit learning of behaviors appropriate to local predator environments. No systematic studies of juvenile glucocorticoids across early development have been conducted for sciurids. Levels of free cortisol are similar in male and female Arctic ground squirrel juveniles (*S. parryii*; ages unknown), but are lower than those of adult males (Boonstra et al., 2001). In yellow-pine chipmunks (*Tamias amoenus*), juvenile (above ground for 4–6 weeks) cortisol and corticosterone levels are indistinguishable from adult levels (Kenagy and Place, 2000; Place and Kenagy, 2000). For Belding's ground squirrels, Morton et al. (1974) reported that juvenile (young-of-the-year) adrenal glands enlarge shortly before hibernation (when animals rapidly accumulate body fat), yet McKeever (1963) did not detect any increase in gland weights at this time. Nunes et al. (2002) found that acute corticosterone responses by juvenile *S. beldingi* are higher a month after emergence compared with the second month aboveground, when young are beginning to gain weight for hibernation. It is likely that basal glucocorticoids of juvenile *S. beldingi* change across development, for example increasing to mobilize energy for emergence or dispersal and decreasing to facilitate body growth (Sapolsky, 1992).

Using a non-invasive fecal hormone sampling procedure, I characterized basal levels of cortisol in juveniles during the initial weeks after natal emergence. I studied juveniles from three locations in California which vary in habitat, visual openness, predator types and anti-predator responses by adults (J.M. Mateo, unpublished data) to determine if juvenile corticoid levels also vary by population as well as by age. In addition, any age-related changes in cortisol could simply be due to stressors experienced by all young, independent of their particular population, such as exposure to novel visual, olfactory and auditory stimuli when first emerging aboveground (Mateo, 1996). I therefore monitored developmental variation in cortisol in captive-born laboratory-housed juveniles which experienced no changes in their exposure to stimuli or in their physical or social environments around the age of emergence.

Methods

Animals and study sites

Belding's ground squirrels are group-living, burrowing rodents found in alpine and subalpine regions of the western United States (Jenkins and Eshelman, 1984). They are socially active above ground between April and August and hibernate the remainder of the year. Each adult female produces one litter annually of 5–8 pups, which is reared for about a month in an underground burrow (the natal burrow). Young first come above ground (emerge) as nearly weaned, 4-week old juveniles. About 1 month after natal emergence, juvenile females establish their own burrow system within 25 m of their natal burrow, whereas juvenile males begin to disperse permanently from their birthplace (Holekamp, 1984). Females live an average of 3.4±0.3 years (up to 12 years); males live 2.5±0.4 years (up to 9 years; Sherman and Morton, 1984; personal observation). The research reported here adheres to standards set forth by the NIH for animal research.

Free-living animals

Field research was conducted during the summers of 2002–2005 at three sites in the eastern Sierra within 100 km of Mammoth Lakes, CA, USA. The Mono County Park site (elevation 1966 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 the vegetation height was never higher than 5 cm. The Lundy Canyon site (2316 m) is a visually closed collection of campsites closely surrounded 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 cm to ~70 cm, and were typically >40 cm high at natal emergence. The Rock Creek Canyon site (Lower Horse Corral; 2834 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 ranged from 1 cm at the beginning and end of the active season to 15 cm high at the peak of wildflower growth. Adults at Lundy Canyon spend more time vigilant and less time feeding than those at Rock Creek or Mono Co. Park. Lundy Canyon adults spend more time alert and exhibit more vigilant responses after hearing alarm calls. Adults at Lundy Canyon also have lower fecal corticoid levels than those at Mono Co. Park and Rock Creek (J.M. Mateo, unpublished. data).

To characterize changes in basal cortisol of free-living juveniles, feces were collected from *S. beldingi* at the three sites when juveniles first emerged

Table 1
Number of juvenile *S. beldingi* sampled each year from each population

	2002		2003		2004		2005	
	Male	Female	Male	Female	Male	Female	Male	Female
Rock Creek	68	69	20	36	31	20	22	19
Lundy Canyon	9	0	10	15	15	23		
Mono Co. Park	15	23	15	22	14	30	16	34

aboveground and again approximately 2 weeks later (Table 1). Data collection was more intensive at Rock Creek, where individually marked juveniles were sampled approximately weekly (for 10–40 days, depending on the length of my field season). Each collection period (here called 'rounds') lasted 2–3 days. Because of other ongoing studies, I was unable to return to Lundy Canyon for a second round of sampling in 2002. In addition, only five juveniles were sampled there in 2005 due to low population density, and thus those data were not included in analyses. After their first fecal sample was collected, juveniles at Rock Creek were marked individually with ear tags (one on each ear; National Band and Tag Co.; Newport, KY USA) or intrascapular PIT tags (Biomark Inc., Boise, ID, USA). Due to time constraints of other studies, juveniles at Mono Co. Park and Lundy Canyon were only individually marked with ear tags in 2005. However, in all years at all sites we clipped some hair from the dorsal region of all animals that were trapped, and thus we were able to avoid sampling animals which had been trapped earlier during that round. Therefore, the analyses did not include samples with artificially elevated corticoid levels due to the stress of trapping and handling, nor did it include multiple samples from an individual within a round. After marking, juveniles were weighed to the nearest gram (in a 2 L drinking pitcher, with pour tab removed, placed on a portable Ohaus balance) and then released at their site of capture. Some juveniles were sampled in more than one round. Of the individually marked *S. beldingi*, across the years 23–46% of juveniles at Rock Creek sampled in Round 1 were also sampled in Round 2, and 28% of Mono Co. Park juveniles sampled in Round 1 in 2005 were also sampled in Round 2.

Captive animals

To determine whether there are developmental changes in glucocorticoids in animals experiencing a constant environment, I studied animals born and reared in captivity in 2003. This research was conducted at the Sierra Nevada Aquatic Research Laboratory (SNARL; University of California Natural Reserve System, near Mammoth Lakes, CA, USA). Pregnant females were live-trapped and housed in a laboratory building at SNARL where they gave birth and reared their young. Each mother and her litter were housed in a nestbox inside a larger cage (details in Mateo and Holmes, 1997). I measured the corticoids of 17 male and 18 female juveniles from 9 litters born to mothers collected at Mono County Park and Lundy Canyon. Sampling occurred once every 3 days, starting at 26 days of age, the approximate age at which juveniles would normally emerge aboveground, and continued for 2 weeks until the age at which alarm-call responses are well formed (Mateo, 1996). Juveniles were individually marked with ear tags. Because of the conclusion of my field season, I was not able to sample some later-born litters at all six time points. Juveniles excreted insufficient feces at earlier ages for analysis. At the end of the studies, animals were released at the mother's point of capture. Survival rates of previously captive animals match those of free-living animals observed in my mark-release studies (unpublished data). Adult females typically regain their territories after returning to their site of capture. Anti-predator behaviors of juveniles born in captivity and released within a couple of months are qualitatively similar to those of field-reared juveniles (Mateo and Holmes, 1999b). In addition, the feeding regimen of captive animals results in higher body weights than those of same-aged, free-living animals, increasing the chances of released juveniles surviving their first hibernation (60% of field-born juveniles do not survive the winter, probably due to insufficient body-fat reserves; Sherman and Morton, 1984; personal observation).

Fecal sampling

I used a non-invasive fecal measure of cortisol, which in adults provides an integrated measure of basal corticoids over the previous 6–12 hr (rather than serum values which would reflect acute responses related to the immediate stress of trapping and handling). Ground squirrels typically defecate while in traps (single-door live traps; Tomahawk Co., Tomahawk, WI, USA) and the fecal pellets fall into the vegetation below the traps. Therefore, for free-living juveniles, within 5 min of an animal trapping itself the trap was moved aside and

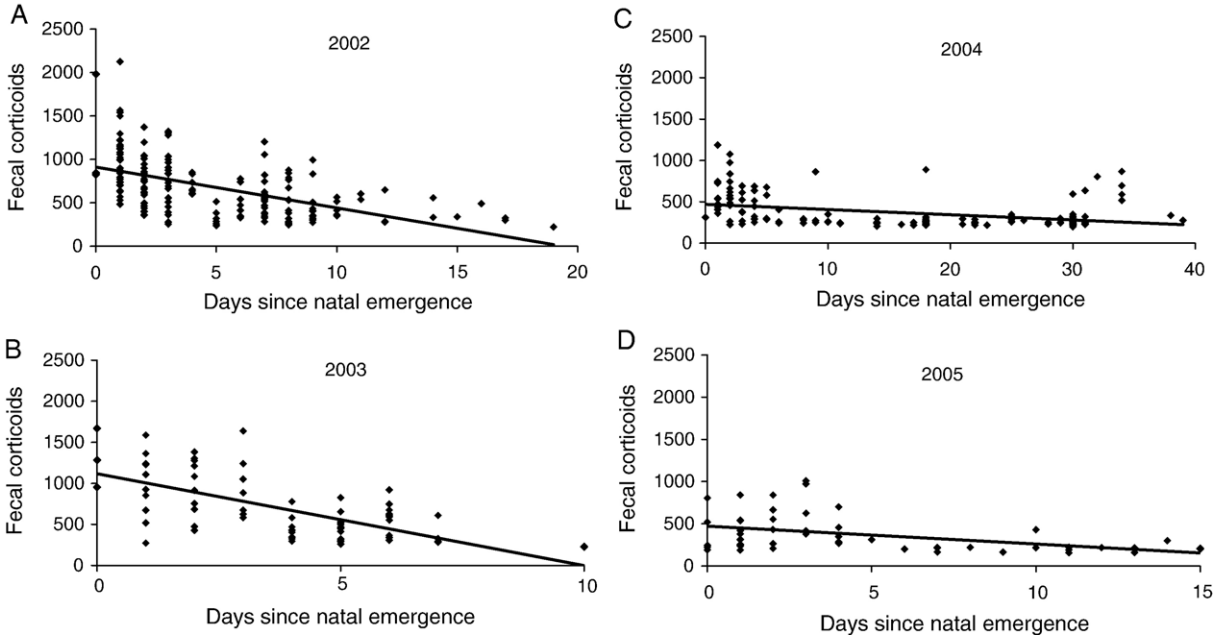

Fig. 1. Correlations between days since natal emergence and fecal corticoids (ng/g dried feces) in juvenile *S. beldingi* at Rock Creek. Unadjusted fecal cortisol levels are shown; analyses were based on log-transformed data. (A) 2002: $n=166$, $r=-0.57$, $P<0.0001$; $y=-0.073\times+6.756$, regression $t=-8.89$, $P<0.0001$. (B) 2003: $n=69$, $r=-0.65$, $P<0.0001$; $y=-0.16\times+7.007$, regression $t=-6.90$, $P<0.0001$. (C) 2004: $n=154$, $r=-0.41$, $P<0.0001$; $y=-0.015\times+6.049$, regression $t=-5.60$, $P<0.0001$. (D) 2005: $n=52$, $r=-0.49$, $P<0.0001$; $y=-0.057\times+6.047$, regression $t=-3.95$, $P<0.0001$.

Table 2
Results of ANOVAs examining changes in cortisol levels by sampling period ('round') in juvenile *S. beldingi* after natal emergence

Population	Year	ANOVA
Rock Creek	2002	$F_{2,177}=33.59$, $P<0.0001$
	2003	$F_{1,67}=30.96$, $P<0.0001$
	2004	$F_{5,14}=16.23$, $P<0.0001$
	2005	$F_{1,48}=1.54$, $P=0.22$
Mono Co. Park	2002	$F_{1,75}=73.49$, $P<0.0001$
	2003	$F_{1,72}=65.49$, $P<0.0001$
	2004	$F_{1,54}=4.35$, $P=0.042$
	2005	$F_{1,60}=8.30$, $P=0.005$
Lundy Canyon	2003	$F_{1,37}=14.93$, $P<0.0001$
	2004	$F_{1,38}=24.17$, $P<0.0001$

Levels were significantly higher in Round 1, which includes samples collected during the first 5 days after natal emergence, compared with subsequent rounds, when sampling occurred approximately every 2 weeks (see Fig. 2). At Rock Creek in 2002 and 2004, juveniles were sampled once a week thereafter, and Bonferroni pairwise comparisons were used for post hoc analyses (Ps <0.01).

Table 3
Results of ANOVAs examining population differences (Rock Creek, Lundy Canyon and Mono Co. Park) in cortisol levels during Round 1 (the first 5 days after emergence) and during Round 2 (approximately 2 weeks later)

Year	Round	ANOVA
2002	1	$F_{2,148}=0.066$, $P=0.94$
	2	$F_{1,111}=27.68$, $P<0.0001$
2003	1	$F_{2,94}=2.45$, $P=0.09$
	2	$F_{2,76}=24.43$, $P<0.0001$
2004	1	$F_{2,94}=2.78$, $P=0.07$
	2	$F_{2,52}=2.399$, $P=0.101$
2005	1	$F_{1,64}=2.68$, $P=0.106$
	2	$F_{1,44}=6.64$, $P=0.013$

Samples from Lundy Canyon were not available for Round 2 in 2002 or for Rounds 1 and 2 in 2005. See Fig. 2 for identification of significant differences between populations.

the pellets collected with clean tweezers and transferred to polypropylene microcentrifuge tubes (Cole Parmer; Vernon Hills, IL). If animals did not immediately defecate, their trap was set inside a clean plastic bucket until defecation (usually <30 min). Captive juveniles were transferred from their nestbox to individual traps placed in clean buckets. In some cases (<5% of samples) animals were held with a gloved hand and their ano-genital area softly massaged until they defecated. All animals were weighed after fecal collection. Tweezers and buckets were cleaned between samples with 70% isopropyl alcohol and at the end of the day with Cide-All® germicidal detergent (Chemifax; Santa Fe Springs, CA). Feces contaminated with urine (visibly wet and/or in a pool of urine) were discarded (see Cavigelli et al., 2005 for information on how urine can alter fecal corticoid levels). Samples were eliminated from the analysis if animals had been trapped or handled during the previous 48 h, because fecal corticoids would still be elevated from any stress of that trapping and handling (Mateo and Cavigelli, 2005). Although in adults fecal metabolites represent an average of circulating hormones during a 6–12 h period prior to defecation (based on ACTH and dexamethasone challenges), circadian variation is still present (Cavigelli et al., 2005; unpublished data) and therefore it is necessary to collect samples at a consistent time of day. Field collections were limited to 0800–1200 h and because of fieldwork in the morning, all fecal collections from captive juveniles occurred between 1500 and 1800 h. Samples were stored immediately at −15°C and then transferred to −80°C at the end of the field season (4–6 weeks later).

Fecal cortisol metabolite assays

Fecal–corticoid metabolites were measured following the methods outlined in Mateo and Cavigelli (2005). Samples were thawed and placed in a drying oven (95°C) for 4–6 h to evaporate the water. For each sample, dried fecal pellets were crushed and mixed together, after which 0.2 g was weighed into a

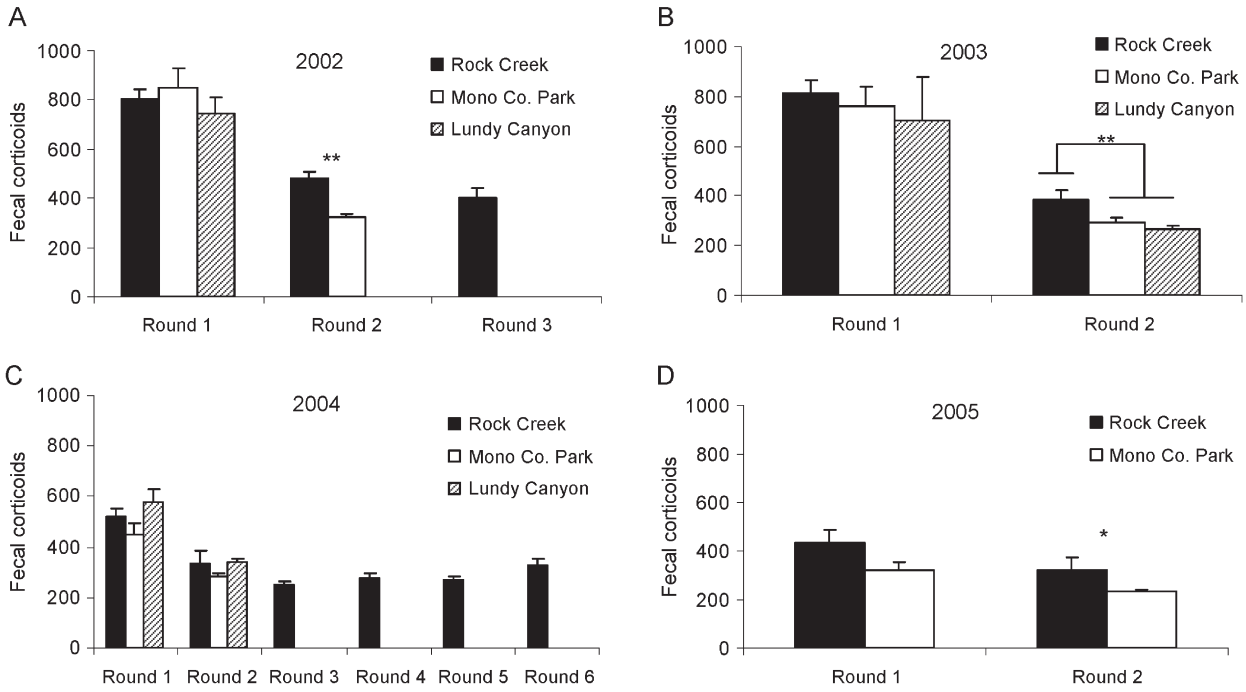

Fig. 2. Patterns of developmental and geographic variation in fecal corticoids (ng/g dried feces+SEM) of free-living juvenile *S. beldingi* from three populations in 2002–2005 (A–D). Unadjusted fecal cortisol levels are shown; analyses were based on log-transformed data. Round 1 represents fecal sampling during the first 5 days after natal emergence and Round 2 occurred approximately 2 weeks later. After Round 2, juveniles were sampled almost weekly at Rock Creek for several weeks in 2002 and 2004. Lines over columns indicate significant population differences within an age group based on Bonferroni pairwise comparisons for significant ANOVAs (*$P<0.05$; **$P<0.01$). See Tables 2 and 3 for ANOVA results.

microcentrifuge tube. Next, 1.5 mL of 80% ethanol was added to each tube, which was briefly vortexed (~3 s) and immediately centrifuged (2500×g, 20 min). Supernatants were reserved and frozen (−80°C) until assayed. Samples were assayed with cortisol solid phase component system ^{125}I-cortisol Corticote® radioimmunoassay kits (MP Biomedicals, Irvine, CA; *S. beldingi* produce both corticosterone and cortisol, but cortisol is the predominant circulating glucocorticoid; Mateo and Cavigelli, 2005) and were assayed in duplicate and re-analyzed if the coefficient of variation between duplicates exceeded 20%. The sensitivity of the assay is 0.07 µg/dL, according to the manufacturer. Two control samples, each made by pooling fecal extracts from 5 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 9 different batches of each pool) of the low and high pools, mean intra- and interassay coefficients of variation for the assays were 9.08% and 11.12%, respectively, for the low pool and 6.19% and 10.90% for the high pool. Fecal corticoid concentrations were divided by the proportion of isotope recoveries, and are expressed as ng/g of dried feces. Cortisol data were log-transformed for normality, and distributions were verified with Kolmogorov–Smirnov tests. Extracted samples were assayed after varying periods in deep storage after extraction (−80°C; 0.25 to 2.5 years), and it is unknown whether *S. beldingi* metabolites degrade during long-term storage (e.g. Khan et al., 2002; Beehner and Whitten, 2004). However, the 2004 and 2005 samples were assayed within 2 months of extraction, so statistical comparisons were made between those years.

Results

Free-living juveniles

Fecal corticoid levels of juveniles at Rock Creek, which were measured weekly, were high after natal emergence and decreased significantly over time (Fig. 1). Specifically, when data were pooled into 5-day categories, cortisol levels during the first five days after emergence were 1.3–2.1 times higher than subsequent days (see Rock Creek data in Table 2; Fig. 2). To determine whether elevated corticoids at natal emergence occurred at other populations, I also sampled juveniles from Mono Co. Park and Lundy Canyon. In all populations, cortisol levels were higher in juveniles sampled during the first several days after natal emergence compared with 2 weeks later (Round 1 versus Round 2; see Table 2; Fig. 2). The data in Figs. 1 and 2 include values from juveniles sampled in more than one round. When the analysis is restricted to juveniles with known identities who were sampled twice, cortisol metabolites also declined significantly from Round 1 to Round 2 (two-tailed paired t test on data pooled across years, t_{88}=9.66, P<0.0001).

If cortisol levels reflect individuals' reactions to stressors, then animals living in sites where adults perceive high predation risk (e.g. Lundy Canyon) might have different cortisol profiles than those living in low predation–pressure sites. Yet there were no differences in fecal cortisol just after natal emergence (Round 1, Table 3). Significant population differences in corticoids were not evident until young had been aboveground for 2 weeks (Round 2, Table 3; Fig. 2). Fecal–corticoid levels were higher in 2004 than in 2005 during Round 1 at Rock Creek ($F_{1,79}$=9.17, P=0.003) and during Round 2 at Mono Co. Park ($F_{1,69}$=16.57, P<0.0001). There were no significant litter or sex differences in fecal–cortisol levels within age groups or within populations (all Ps>0.50).

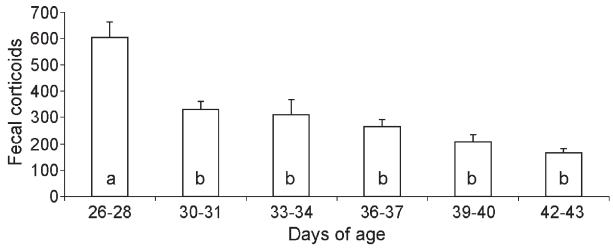

Fig. 3. Unadjusted mean fecal corticoids (ng/g dried feces+SEM) of 12 captive-born juvenile *S. beldingi.* Different letters inside columns indicate significant age differences based on a repeated-measures ANOVA of log-transformed data.

Captive juveniles

As was observed in free-living juveniles, fecal corticoids of captive-reared young were significantly higher at 26–28 days, the age at which juveniles would emerge in the field, compared with all subsequent ages (12 juveniles sampled at each time point $F_{5,55}$=9.41, P<0.0001, Fig. 3; 35 juveniles sampled at the first two time points $F_{1,35}$=36.17, P<0.0001; 603.07 ng/g+59.78 and 329.03 ng/g+31.04, respectively). No significant litter, population or sex differences were observed (all Ps>0.50).

Discussion

Juvenile *S. beldingi* exhibit age-related changes in fecal corticoids after they emerge aboveground from their natal burrows for the first time at about 4 weeks of age. Specifically, corticoid levels are elevated during the first 5 days after emergence compared with subsequent days (Figs. 1 and 2). During these initial days, juveniles are learning important survival strategies, including new diets, anti-predator responses and the locations of escape burrows in their mother's territory. Learning whether and how to respond to alarm and non-alarm calls occurs quickly during these first 5 days, whereas during the following month their behaviors are fine-tuned but do not change qualitatively (Mateo, 1996). The elevated cortisol observed at emergence might facilitate acquisition of anti-predator behaviors (Lupien and McEwen, 1997). Indeed, moderately elevated cortisol significantly improves associative and spatial learning in captive juvenile *S. beldingi* (unpublished data).

Knowledge of glucocorticoid profiles across development is essential for understanding how stressors can influence young animals' behaviors, especially those related to survival. To date, most research on adrenal effects on behavioral and physiological development has used captive animals which can be repeatedly bled. For example, corticosterone levels of young laboratory rats (*Rattus norvegicus*) increase to adult levels by the age of weaning after a stress hyporesponsive period shortly after birth when levels are extremely low (Henning, 1978; Sapolsky and Meaney, 1986). Corticosterone levels of captive American kestrel (*Falco sparverius*) nestlings increase from very low baseline after hatching to adult levels by 22 days of age (Love et al., 2003). Similarly, corticosterone is significantly elevated during the fledgling period in captive canaries (*Serinus canaria*), compared with nestling and adult levels (Schwabl, 1999). Humans (*Homo*

sapiens) also show changes in glucocorticoids as a function of developmental transitions, with cortisol elevated during the first 15 days after birth, and remaining low until puberty (Elmlinger et al., 2002). Little is known about HPA development and adrenal activity in free-living animals across early development, although recently developed protocols for non-invasive monitoring of glucocorticoids make repeated sampling of animals in the field more practical. In wild baboons (*Papio cynocephalus*), fecal corticoids remain fairly constant during the juvenile period, with female but not male levels decreasing in the year prior to sexual maturation. This sex difference might reflect differences in social development since female rank is largely stable by this age (Gesquiere et al., 2005). Frigerio et al. (2001) studied fecal corticosterone of hand-reared, unrestrained greylag geese (*Anser anser*) from hatching until fledging. Corticoids declined during the first 20 days, and remained low for the remainder of the period. The authors suggest that elevated levels after hatching promote imprinting, and that lower levels later facilitate body growth. A similar mechanism might operate in *S. beldingi*, with elevated levels at emergence to promote rapid learning, and lower levels thereafter as juveniles gain weight for their first hibernation (see Sapolsky, 1992) and future studies could quantify glucose concentrations across sites throughout the active season (sensu Boonstra and McColl, 2000).

In addition to age-related changes in glucocorticoids, variation within species as a function of strain or environment has been observed in captive and free-living animals. In laboratory rodents, differences among strains have been observed in basal glucocorticoid levels as well as in stress-induced responses (e.g. Sakaguchi et al., 1984; Anisman et al., 1998). Birds commonly show population differences in response to stressors (e.g. climate at different latitudes or in different habitats; Wingfield et al., 1997). I also found population differences in juvenile (Table 3; Fig. 2) and adult fecal corticoids (unpublished data). Note that differences in fecal cortisol might reflect differences in HPA regulation rather than ongoing differences in exposure to stressors, perhaps through pre-emergent tuning of the HPA axis (e.g. Catalani, 1997; see also Mateo and Holmes, 1999a). The three sites studied here differed in ground-squirrel density, predator types and habitat quality (e.g. availability of refugia, visibility of approaching predators). Yet despite these differences, fecal corticoids did not differ significantly as a function of location after emergence (Round 1, Table 3), and it is unclear how the near-significant P values would change with even larger sample sizes. Significant population differences in cortisol levels were not evident until 2 weeks after emergence (Round 2, Table 3). Fecal corticoids were significantly higher in 2004 than 2005, but only at Rock Creek in Round 1 and Mono Co. Park in Round 2. Without accompanying behavioral data, it is difficult to interpret these differences or explain why they were not uniform across populations and developmental periods.

That cortisol appears to be elevated equally in all three populations after emergence could simply be an effect of new environmental input. Juveniles spend the first 4 weeks or so of their lives below ground in a dark, quiet burrow, interacting only with their mother and littermates (although on rare occurrence a predator or infanticidal *S. beldingi* might enter the burrow system). After emergence, they are exposed to bright sunlight and other novel visual input, sounds and odors from conspecifics, heterospecifics and the physical environment, and encounters with other conspecifics. Nonetheless, juveniles born and reared in captivity, and thus experiencing constant environmental input, also show elevated cortisol metabolites at the age at which they would be emerging in the field and lower levels during the following two weeks (Fig. 3). Although both free-living and captive juveniles might be experiencing changes in social interactions (e.g. playing with littermates; Holmes and Mateo, 1998), these would not be unpredictable stressors, and thus are unlikely to account for the increase in corticoids after emergence.

My results demonstrate that the elevated corticoids of free-living juvenile *S. beldingi* at an important developmental milestone are not simply due to an individual's stressful experiences but instead reflect modulation of stress hormones across early development. This spontaneous elevation, not triggered by external events, could have evolved originally for other reasons, such as energy mobilization to promote emergence from natal burrows, similar to the glucocorticoid changes associated with fledging, dispersal or migration-related activities in birds (e.g. Heath, 1997; Belthoff and Dufty, 1998; Piersma et al., 2000; cf. results with spotted hyenas, *Crocuta crocuta*; Holekamp and Smale, 1998; and muriquis, *Brachyteles arachnoids*; Strier and Ziegler, 2000). However, the evolutionary maintenance of higher levels at emergence might also facilitate rapid learning and memory formation. One interpretation of my results is that natural selection has favored a plastic or open program (sensu Mayr, 1974) for anti-predator behaviors, as *S. beldingi* inhabit a range of predator environments and thus there is no single optimal response repertoire (Mateo, 1996; Mateo and Holmes, 1999b). In contrast, cortisol secretion around emergence follows a closed program, with levels determined by an animal's age rather than the particular environment into which it emerges. This is counter-intuitive at first, because one might expect juveniles to have population-specific cortisol levels according to local predation pressure, which might allow them to regulate glucose and cardiovascular tone for escaping from predators and for foraging. These two behaviors must be carefully balanced after emergence because juveniles are transitioning to solid foods and are vulnerable to the increasing number of predators they attract. However, basal cortisol appears to be unrelated to the local environment at natal emergence (although acute cortisol responses may differ between populations), and this might be because very high cortisol can impair learning (e.g. Lupien and McEwen, 1997; unpublished data). This upper limit on cortisol secretion during early development could be analogous to the stress hyporesponsive period seen in rats and perhaps humans (Sapolsky and Meaney, 1986; Gunnar and Donzella, 2002). Our current work is investigating how these uniformly elevated levels of cortisol at emergence facilitate rapid acquisition of anti-predator behaviors during this dangerous developmental period.

Glucocorticoids are intricately involved in almost all aspects of an individual's life, including its growth and maintenance, daily rhythms, cognitive functioning and responses to stressors. Yet little is known about the functions of adrenal hormones across development, particularly age-related changes associated with major transitions such as weaning, fledging, dispersal or puberty. In addition, early experiences can have lasting behavioral and physiological effects which can even be transmitted non-genetically across generations (e.g. Meaney, 2001). Because these early experiences can influence survival and reproduction, it is important that we gain an understanding of how social and ecological variables influence HPA functioning. The results presented here suggest that stress hormones of *S. beldingi* might influence (and might be influenced by) natural selection, with cortisol levels elevated at natal emergence, perhaps to facilitate movement through and emergence from the burrow system and, secondarily, to facilitate learning, whereas levels are population-specific at later ages, likely corresponding to local levels of predation pressure. With the increasing application of non-invasive hormonal sampling, more researchers can investigate ecologically relevant age, sex or population effects on adrenal functioning.

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References

Abbott, D.H., Keverne, E.B., Bercovitch, F.B., Shively, C.A., Mendoza, S.P., Saltzman, W., Snowdon, C.T., Ziegler, T.E., Banjevic, M., Garland, T.J., Sapolsky, R.M., 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Horm. Behav. 43, 67–82.

Anisman, H., Zaharia, M.D., Meaney, M.J., Merali, Z., 1998. Do early-life events permanently alter behavioral and hormonal responses to stressors? Int. J. Dev. Neurosci. 16, 149–164.

Apanius, V., 1998. Stress and immune defense. Adv. Stud. Behav. 27, 133–153.

Bachman, G.C., 1993. The effect of body condition on the trade-off between vigilance and foraging in Belding's ground squirrels. Anim. Behav. 46, 233–244.

Beehner, J.C., Whitten, P.L., 2004. Modifications of a field method for fecal steroid analysis in baboons. Physiol. Behav. 82, 269–277.

Belthoff, J.R., Dufty, A.M., 1998. Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. Anim. Behav. 55, 405–415.

Boonstra, R., 2005. Equipped for life: the adaptive role of the stress axis in male mammals. J. Mammal. 86, 236–247.

Boonstra, R., McColl, C.J., 2000. Contrasting stress response of male arctic ground squirrels and red squirrels. J. Exp. Zool. 286, 390–404.

Boonstra, R., Hubbs, A.H., Lacey, E.A., McColl, C.J., 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. Can. J. Zool. 79, 49–58.

Buchanan, K.L., 2000. Stress and the evolution of condition-dependent signals. Trends Ecol. Evol. 15, 156–160.

Catalani, A., 1997. Neonatal exposure to glucocorticoids: long-term endocrine and behavioral effects. Dev. Brain Dysfunct. 10, 393–404.

Cavigelli, S.A., 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. Anim. Behav. 57, 935–944.

Cavigelli, S.A., Monfort, S.L., Whitney, T.K., Mechref, Y.S., Novotny, M., McClintock, M.K., 2005. Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms. J. Endocrinol. 184, 153–163.

Creel, S.R., 2001. Social dominance and stress hormones. Trends Ecol. Evol. 16, 491–497.

Elmlinger, M.W., Kuhnel, W., Ranke, M.B., 2002. Reference ranges for serum concentrations of lutropin (LH), follitropin (FSH), estradiol (E2), prolactin, progesterone, sex hormone-binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and ferritin in neonates, children and young adults. Clin. Chem. Lab. Med. 40, 1151–1160.

Frigerio, D., Moestl, E., Kotrschal, K., 2001. Excreted metabolites of gonadal steroid hormones and corticosterone in greylag geese (*Anser anser*) from hatching to fledging. Gen. Comp. Endocrinol. 124, 246–255.

Gesquiere, L.R., Altmann, J., Khan, M.Z., Couret, J., Yu, J.C., Endres, C.S., Lynch, J.W., Ogola, P., Fox, E.A., Alberts, S.C., Wango, E.O., 2005. Coming of age: steroid hormones of wild immature baboons (*Papio cynocephalus*). Am. J. Primatol. 67, 83–100.

Goymann, W., East, M.L., Wachter, B., Honer, O.P., Möstl, E., Van't Hof, T.J., Hofer, H., 2001. Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. Proc. R. Soc. Lond., B Biol. Sci. 268, 2453–2459.

Gunnar, M.R., Donzella, B., 2002. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 27, 199–220.

Heath, J., 1997. Corticosterone levels during nest departure of juvenile American kestrels. Condor 99, 806–811.

Henning, S.J., 1978. Plasma concentrations of total and free corticosterone during development in the rat. Am. J. Physiol. 235, E451–E456.

Holekamp, K.E., 1984. Natal dispersal in Belding's ground squirrels (*Spermophilus beldingi*). Behav. Ecol. Sociobiol. 16, 21–30.

Holekamp, K.E., Smale, L., 1998. Dispersal status influences hormones and behavior in the male spotted hyena. Horm. Behav. 33, 205–216.

Holmes, W.G., Mateo, J.M., 1998. How mothers influence the development of litter-mate preferences in Belding's ground squirrels. Anim. Behav. 55, 1555–1570.

Hubbs, A.H., Millar, J.S., Wiebe, J.P., 2000. Effect of brief exposure to a potential predator on cortisol concentrations in female Columbian ground squirrels (*Spermophilus columbianus*). Can. J. Zool. 78, 578–587.

Jenkins, S.H., Eshelman, B.D., 1984. *Spermophilus beldingi*. Mamm. Species 221, 1–8.

Jennings, D.H., Painter, D.K., Moore, M.C., 2004. The role of the adrenal gland in early posthatching differentiation of alternative male phenotypes in the tree lizard. Gen. Comp. Endocrinol. 135, 81–89.

Kenagy, G.J., Place, N.J., 2000. Seasonal changes in plasma glucocorticoids of free-living female yellow-pine chipmunks: effects of reproduction and capture and handling. Gen. Comp. Endocrinol. 117, 189–199.

Khan, M.Z., Altmann, J., Isani, S.S., Yu, J., 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen. Comp. Endocrinol. 128, 57–64.

Koolhaas, J.M., Korte, S.M., Boer, S.F., Van Der Vegt, B.J., Van Renen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping

styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.
Kotrschal, K., Hirschenhauser, K., Möstl, E., 1998. The relationship between social stress and dominance is seasonal in greylag geese. Anim. Behav. 55, 171–176.
Lima, S.L., 1998. Stress and decision making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. Adv. Study Behav. 27, 215–290.
Love, O.P., Bird, D.M., Shutt, L.J., 2003. Corticosterone levels during post-natal development in captive American kestrels (*Falco sparverius*). Gen. Comp. Endocrinol. 130, 135–141.
Lupien, S.J., McEwen, B.S., 1997. The acute effects of corticosteroids on cognition: integration of animal and human model studies. Brain Res. Rev. 24, 1–27.
Mateo, J.M., 1996. The development of alarm-call response behaviour in free-living juvenile Belding's ground squirrels. Anim. Behav. 52, 489–505.
Mateo, J.M., Cavigelli, S.A., 2005. A validation of extraction methods for non-invasive sampling of glucocorticoids in free-living ground squirrels. Physiol. Biochem. Zool. 78, 1069–1084.
Mateo, J.M., Holmes, W.G., 1997. Development of alarm-call responses in Belding's ground squirrels: the role of dams. Anim. Behav. 54, 509–524.
Mateo, J.M., Holmes, W.G., 1999a. How rearing history affects alarm-call responses of Belding's ground squirrels (*Spermophilus beldingi*, Sciuridae). Ethology 105, 207–222.
Mateo, J.M., Holmes, W.G., 1999b. Plasticity of alarm-call response development in Belding's ground squirrels (*Spermophilus beldingi*, Sciuridae). Ethology 105, 193–206.
Mayr, E., 1974. Behavior programs and evolutionary strategies. Am. Sci. 62, 650–659.
McEwen, B.S., Sapolsky, R.M., 1995. Stress and cognitive function. Curr. Opin. Neurobiol. 5, 205–216.
McGaugh, J.L., 2000. Memory-A century of consolidation. Science 287, 248–251.
McKeever, S., 1963. Seasonal changes in body weight, reproductive organs, pituitary, adrenal glands, thyroid gland, and spleen of the Belding ground squirrel (*Citellus beldingi*). Am. J. Anat. 113, 153–173.
Meaney, M.J., 2001. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu. Rev. Neurosci. 24, 1161–1192.
Morton, M.L., Sherman, P.W., 1978. Effects of a spring snowstorm on behavior, reproduction, and survival of Belding's ground squirrels. Can. J. Zool. 56, 2578–2590.
Morton, M.L., Maxwell, C.S., Wade, C.E., 1974. Body size, body composition, and behavior of juvenile Belding ground squirrels. Great Basin Nat. 34, 121–134.
Murie, J.O., Boag, D.A., 1984. The relationship of body weight to overwinter survival in Columbian ground squirrels. J. Mammal. 65, 688–690.
Nunes, S., Muecke, E.M., Holekamp, K.E., 2002. Seasonal effects of food provisioning on body fat, insulin, and corticosterone in free-living juvenile Belding's ground squirrels (*Spermophilus beldingi*). Can. J. Zool. 80, 366–371.
Piersma, T., Reneerkens, J., Ramenofsky, M., 2000. Baseline corticosterone peaks in shorebirds with maximal energy stores for migration: a general preparatory mechanism for rapid behavioral and metabolic transitions? Gen. Comp. Endocrinol. 120, 118–126.
Place, N.J., Kenagy, G.J., 2000. Seasonal changes in plasma testosterone and glucocorticoids in free-living male yellow-pine chipmunks and the response to capture and handling. J. Comp. Physiol., B 170, 245–251.
Sakaguchi, A., LeDoux, J.E., Sved, A.F., Reis, D.J., 1984. Strain differences in fear between spontaneously hypertensive and normotensive rats is mediated by adrenal cortical hormones. Neurosci. Lett. 46, 59–64.
Sands, J.L., Creel, S., 2004. Social dominance, aggression and fecal glucocorticoid levels in a wild population of wolves, *Canis lupus*. Anim. Behav. 67, 387–396.
Sapolsky, R.M., 1992. Neuroendocrinology of the stress–response. In: Becker, J.B., et al. (Ed.), Behavioral Endocrinology. MIT Press, Cambridge, MA, pp. 287–324.
Sapolsky, R.M., Meaney, M.J., 1986. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. Brain Res. Rev. 11, 65–76.
Schwabl, H., 1999. Developmental changes and among-sibling variation of corticosterone levels in an altricial avian species. Gen. Comp. Endocrinol. 116, 403–408.
Sherman, P.W., 1982. Infanticide in ground squirrels. Anim. Behav. 30, 938–939.
Sherman, P.W., 1985. Alarm calls of Belding's ground squirrels to aerial predators: nepotism or self-preservation? Behav. Ecol. Sociobiol. 17, 313–323.
Sherman, P.W., Morton, M.L., 1984. Demography of Belding's ground squirrels. Ecology 65, 1617–1628.
Spencer, K.A., Wimpenny, J.H., Buchanan, K.L., Lovell, P.G., Goldsmith, A.R., Catchpole, C.K., 2005. Developmental stress affects the attractiveness of male song and female choice in the zebra finch (*Taeniopygia guttata*). Behav. Ecol. 58, 423–428.
Strier, K.B., Ziegler, T.E., 2000. Lack of pubertal influences on female dispersal in muriqui monkeys, *Brachyteles arachnoides*. Anim. Behav. 59, 849–860.
Wingfield, J.C., 2004. Control of behavioural strategies for capricious environments. Anim. Behav. 66, 807–816.
Wingfield, J.C., Breuner, C.W., Jacobs, J., 1997. Corticosterone and behavioral responses to unpredictable events. In: Harvey, S., Etches, R.J. (Eds.), Perspectives in Avian Endocrinology. . Journal of Endocrinology. Limited, Bristol, pp. 267–278.
Wingfield, J.C., Maney, D.L., Breuner, C., Jacobs, J.D., Lynn, S., Ramenofsky, M., Richardson, R.D., 1998. Ecological bases of hormone–behavior interactions: the "emergency life history stage". Am. Zool. 38, 191–206.