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Antipredator responses of California ground squirrels to rattlesnakes and rattling sounds: the roles of sex, reproductive parity, and offspring age in assessment and decision-making rules

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Abstract California ground squirrels (*Spermophilus beecheyi*) and northern Pacific rattlesnakes (*Crotalus viridis oreganus*) have an adversarial relationship. Adults are partially protected by venom resistance and harass rattlesnakes in part to defend their more vulnerable offspring. Larger, warmer snakes are more dangerous than smaller colder snakes, and in escalated conflict squirrels could benefit from risk assessment strategies. Rattlesnakes often rattle at harassing squirrels and rattling sounds produce cues related to body size and temperature. In study 1 we played back rattling sounds from snakes that varied in dangerousness and evaluated the roles of sex and parity in squirrel risk assessment strategies. In general, squirrels tail flagged and stood bipedally more, and were slower to reapproach the playback speaker following playbacks of rattling sounds from more dangerous snakes. In comparison with males and nonmothers, mothers were most responsive to rattling sounds and more sensitive to variation in snake dangerousness. Mothers tail flagged more than males and nonmothers,

and this behavior tracked variation in snake dangerousness most closely, perhaps reflecting the effects of snake size and temperature on pup vulnerability. These findings suggest that many aspects of squirrel antisnake behavior are governed by their effects on descendant kin. In study 2 we tested the effects of offspring age on mothers' responses to live rattlesnakes and rattling sounds. According to the offspring value hypothesis, mothers should take more risks in defense of older offspring because they are more likely to survive to reproductive age. By contrast, under the offspring vulnerability hypothesis, older offspring are less vulnerable to predators and thus mothers should take fewer risks. Risk-taking, as measured by behaviors that bring the squirrel close to the snake's strike range, was either unaffected by or negatively correlated with offspring age. Thus, our findings suggest that whereas offspring value is unimportant in squirrel antisnake behavior, offspring vulnerability may affect maternal defense. We suggest that offspring vulnerability in mammals, in comparison with birds, may play a larger role in parental defense against predators.

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Introduction

Parental care plays an important role in offspring survival and parental fitness, but also often entails immediate and long-term costs that reduce the ability of parents to invest in future offspring (Clutton-Brock 1991). Costs incurred in caring for current offspring must be balanced against any effects on future prospects. Parental defense of offspring against predators is a form of parental care that can have immediate and profound costs for parents (Andersson et al. 1980; Montgomerie and Weatherhead 1988). Although parents often do defend their young against predators, risk-sensitivity governs many of their actions. Behaviors that bring the parent close to the

predator or involve harassment, while more effective at deterring the predator, come at the price of higher risk.

Parental defense of offspring has received a great deal of theoretical and empirical attention in birds (see Montgomerie and Weatherhead 1988; Michl et al. 2000), but comparatively little among researchers of mammals. Offspring defense may be defined as "behavior that decreases the probability that a predator will harm offspring while simultaneously increasing the probability of injury or death to the parent" (Montgomerie and Weatherhead 1988). Additional costs incurred from offspring defense include time, energy, and opportunity costs (Clutton-Brock 1991). Such antipredator activities may encourage the predator to move on by advertising the potential costs entailed in further pursuit of the offspring protected by a vigilant and/or aggressive parent (Curio 1978; Curio and Regelman 1985; Randall and Matocq 1997). It may also serve to recruit mobbers (Owings and Coss 1977; Tamura and Yong 1993; Winkler 1994), elevate vigilance in nearby conspecifics to help maintain an "early warning system" (Owings et al. 1986; Loughry and McDonough 1988; Hersek and Owings 1993), or communicate risk to offspring (Blumstein et al. 1997).

Aggressive confrontation of predators is more likely when the target of the predator is the offspring (Archer 1988). First, offspring may not be able to evade the predator as effectively as adults, thus eliminating escape as a tactic if the parent is to protect the offspring. Second, parents may be less vulnerable to the predator and activities such as close approach and harassment may be less risky. Indeed most cases of escalated antipredator behavior are seen when parents are defending offspring against predators or infanticidal conspecifics (e.g., Ostermeyer 1983; Curio and Regelman 1985; Archer 1988; Hennessy and Owings 1988; Clutton-Brock 1991), and the degree of escalation is strongly influenced by factors related to offspring vulnerability and reproductive value to the parent (Andersson et al. 1980; Montgomerie and Weatherhead 1988). Paternal care, and thus defense of young, is predicted to be rare in many mammals for two reasons (Trivers 1972): (1) where females mate with more than one male the certainty of paternity is in question; and (2) the female's spatial association with the offspring at the time of birth predisposes her to provide parental care.

Here we examine the roles of sex, parity, and offspring value and vulnerability in California ground squirrel antipredator responses to rattlesnakes and their rattling sounds. In previous studies we have shown that these squirrels possess sophisticated risk assessment strategies for dealing with northern Pacific rattlesnakes (Rowe and Owings 1978; Swaisgood et al. 1999a, 1999b). Both snake and squirrel are capable of inflicting injury on one another and squirrel-snake encounters resemble progressive escalation, probing and assessment seen in intraspecific contests (Enquist et al. 1990). Ground squirrel blood contains proteins that bind and neutralize rattlesnake venom, affording some—but not complete—protection against the snake's most dangerous weapon (Poran et al. 1987). Adults confront, harass and occasionally attack

rattlesnakes, provoking the snake to rattle, sham strike or envenomate the squirrel (Owings and Coss 1977; Coss and Owings 1985). Although squirrel pups also possess venom resistance, the snake's venom is sufficient to overwhelm the limited immunity conferred by their small serum volume (Poran and Coss 1990), and rattlesnakes eat primarily young squirrels (Fitch 1949). One function of rattlesnake harassment by adults, therefore, appears to be protection of vulnerable young (Hennessy and Owings 1988; Hersek and Owings 1993; Swaisgood et al. 1999a).

Rattlesnake dangerousness covaries with snake size and body temperature (Rowe and Owings 1990). Large snakes strike farther, faster, and hold on longer than small snakes, and warmer snakes are capable of striking faster and more accurately. Ground squirrels take fewer risks and invest more time in monitoring or harassing from a safe distance when confronting large rather than small rattlesnakes (Swaisgood et al. 1999a). In addition to visual cues of body size, physical and physiological constraints of rattlesnake rattling divulge cues of both body size and temperature (Rowe and Owings 1996). Rattling by larger snakes produces sounds of lower pitch and higher amplitude, an incidental byproduct of the larger diameter of their rattle segments and their greater tail-shaker muscle mass. Warm snakes shake their tails faster and more forcefully, producing louder sounds with higher click rates. Visual assessment of snake body size may be difficult, especially in dark burrows or thick vegetation, since ground squirrels do not always detect rattlesnakes even at close range under relatively optimal observational conditions (Hennessy and Owings 1988; Poran and Coss 1990). Body temperature affords few visual cues. Thus, squirrels may need to rely on these acoustic cues to assess snake dangerousness, and our previous work indicates that they do (Swaisgood et al. 1999b).

We have documented a number of patterns in ground squirrel antipredator behavior that reflect variation in parental motivation (Owings and Morton 1998). *Spermophilus beecheyi* females mate with multiples males, conferring low certainty of paternity (Boellstorff et al. 1994), and thus males are predicted to provide little in the way of paternal care. In agreement with this prediction, antipredator calls to snakes are given predominantly by mothers (Swaisgood et al. 1999a). Similarly, males engage in antisnake tail flagging in a self-interested way, whereas females tail flag in a manner which potentially tracks changing vulnerability of offspring (Hersek and Owings 1993). In interactions with rattlesnakes, maternal females spent significantly more time and effort in antisnake activity than did males or nonmothers (Swaisgood et al. 1999a). In contrast to predictions from theoretical models (Andersson et al. 1980; Montgomerie and Weatherhead 1988), however, females with pups did not engage in more risk-taking (in the sense of remaining closer to the snake) than other squirrels. Perhaps females must mitigate risk because serious injury could compromise their ability to care for the entire litter (cf. Montgomerie and Weatherhead 1988).

It is not only the presence of offspring that is related to parental defense: several parental and offspring characteristics also influence the costs and benefits of defense (Andersson et al. 1980; Montgomerie and Weatherhead 1988; Clutton-Brock 1991). Among these, the value of offspring to parents should increase with offspring age because their probability of surviving to reproductive age increases. This is true because young are closer to reproductive maturity and the age-specific mortality rate decreases with age. As offspring age they also become larger, stronger, and more competent at evading or defending themselves against predators (or in the case of birds, less vulnerable to overheating or hypothermia while the parents are away from the nest dealing with the predator), and parental defense has a correspondingly smaller effect on the probability of offspring survival, leading to the prediction of less parental defense. That is, decreasing offspring vulnerability and increasing offspring value generate conflicting predictions for the effects of offspring age. In birds numerous studies are consistent with the offspring value hypothesis (Andersson et al. 1980; Greig-Smith 1980; Patterson et al. 1980; Montgomerie and Weatherhead 1988; Michl et al. 2000; Pavel and Bures 2001) and a few offer support for the vulnerability hypothesis (Bures and Pavel 1997; Michl et al. 2000). There are a few tests of these hypotheses in other species (e.g., fish: Pressley 1981; Coleman et al. 1985; insects: Tallamy 1982), but strangely these hypotheses are rarely addressed in the literature on antipredator defense of young in mammals. In ground squirrels, pup vulnerability to rattlesnakes declines rapidly shortly after emergence from the burrow, as pups attain sufficient body size to neutralize a larger proportion of venom (Poran and Coss 1990) and become physically and behaviorally competent at dealing with snakes. Thus, one might predict that offspring vulnerability is more important than value in determining parental defense decision rules in this species.

Here we use acoustic playbacks of rattling sounds and presentations of live rattlesnakes to test three general hypotheses examining the role of parenthood in antisnake responses of California ground squirrels.

1. Do mothers respond more strongly to acoustic cues indicating the presence of a rattlesnake than do males and nonmothers?
2. Are mothers more sensitive to risk-related variation in rattle-borne cues than are males and nonmothers? Specifically, we predict that mothers will more strongly differentiate between control tones versus rattling cues, and rattling sounds from warm versus cold and large versus small rattlesnakes. Mothers should be more sensitive to risk variation because they typically remain in proximity to rattlesnakes longer than males and nonmothers and thus are exposed to more cumulative risk (Swaigood et al. 1999a). Also, differential risk posed by large versus small and warm versus cold snakes may be even greater for her offspring. For example, large snakes may also be

more difficult to expel because squirrels take fewer risks in harassing them (Swaigood et al. 1999a) and have only been seen to inflict serious injuries on small snakes (Hersek 1990; unpublished observations). The same arguments probably hold true for warm versus cold snakes. In addition to being less capable of striking and killing pups (Greenwald 1974; Rowe and Owings 1990), small or cold snakes may be less likely to engage in active hunting. Thus, a mother who loses track of a large warm snake or fails to induce it to move out of her home burrow area may suffer greater losses among her offspring, making this assessment task more critical for mothers than males and nonmothers.

3. Does antisnake risk-taking by mothers change with offspring age? An increase in risky behavior as offspring age is consistent with the offspring value hypothesis, while a decrease in risky behavior offers support for the offspring vulnerability hypothesis. Specifically, we predict changes in maternal behavior most closely related to risk, that is, behaviors that bring the squirrel closer to the snake's strike range.

Methods

Study 1: playback of rattling sounds

The study site and general methods are described in detail in Swaigood et al. (1999b). This research was conducted at an established field site at Camp Ohlone in the coastal foothills of northern California, where both California ground squirrels (*S. beecheyi*) and northern Pacific rattlesnakes (*Crotalus viridis oreganus*) are plentiful. Playback trials used in this study were with six males, six nonmaternal females and six maternal females. All squirrels were marked with dye for individual recognition. We located home burrows for each subject by observing their first emergence at dawn. We do not have breeding data for these subjects at the time the study was conducted; however, we do know a great deal about the mating system for squirrels at this specific site, where population density is very high, home ranges overlap extensively, and burrows are close together. The mating system is characterized as overlap promiscuity, wherein solitary individuals mate with multiple partners with overlapping home ranges (Boellstorff et al. 1994). Females mate with an average of 6.7 males during an estrous period lasting 1 day. Although individual males have not been followed for an entire mating season, most males also mate with several different females (our preliminary data suggest at least three and probably many more). All of the males included in this study had established home ranges overlapping numerous female ranges, and therefore ample opportunity to mate. Because most matings occur with females whose home ranges overlap with the male's, the playbacks described below were almost certainly conducted with males that had mated and had potentially sired pups that resided in nearby nursery burrows. Female status was determined by evidence of lactation and later confirmed by the presence of pups, but prior reproductive history is not known reliably for female subjects.

Six different sounds were played back to each subject: rattles from (1) small cold snakes (55 dB), (2) large cold snakes (55 dB), (3) small warm snakes (75 dB), (4) large warm snakes (75 dB), (5) soft control tones (55 dB), and (6) loud control tones (75 dB). We used two small snakes, weighing 21 g and 58 g, and two large snakes, weighing 560 g and 605 g, to generate the recordings. Snakes were recorded when both warm (35° C) and cold (10° C). These body sizes and temperatures were chosen because they

reflect two ends of the natural continuum of risk posed by snakes varying with regard to these factors (Rowe and Owings 1990). Control tones were harmonically structured to cover the same frequency bandwidth found in rattles. Cold snake differed from warm snake rattle playbacks with regard to amplitude and “click rate” (warm snakes shake their tails faster and more vigorously than cold snakes: Rowe and Owings 1996). Only dominant frequency (pitch) differed between large and small snake rattle playbacks. All of these acoustic cues of snake dangerousness (amplitude, pitch, click rate) are easily distinguished by the untrained human ear.

We placed the playback speaker inside a trap approximately 95 cm from the entrance to the squirrel’s home burrow. Bushes were attached to either side of the trap to hide the speaker and cultivate the possibility that a snake may be there hiding. To draw the squirrel to the vicinity we placed a few oats (scented with rattlesnake odor) about 40 cm from the speaker. Squirrels readily approach these traps and regularly return to them looking for oats. When the squirrel arrived at this location, we played back one of the sounds for 8 s and recorded its response for 10 min on videotape. Each subject received all six playbacks, with the order balanced such that each sound was played back an equal number of times during trials 1–6. Playback trials were separated by at least 24 h and no more than 5 days and the time span of the six trials was similar across all subjects.

We selected three non-redundant antisnake behaviors known to be most sensitive to rattling sounds, each of which measures a different dimension of responsiveness (Swaigood et al. 1999b).

1. Hesitancy to reapproach the speaker is the total amount of time the squirrel spent returning to the oats in front of the speaker after moving away following the playback. This measure is a useful index of caution displayed by squirrels returning to an area presumed to contain a rattlesnake (in the case of rattle playbacks). This is the best measure of risk-averse behavior because a squirrel that proceeds too rapidly is more likely to suffer the costs of stumbling upon a cryptic snake. Also, other than biting the snake, approaching the snake is the behavior most likely to provoke rattlesnake striking (Towers and Coss 1990).
2. As a measure of signaling activity, we recorded the total number of side-to-side tail-flag cycles during the reapproach. Tail flagging during this period is most sensitive to the playback sounds because squirrels are most clearly directing their behavior toward the playback stimulus at this time and because squirrels also engage in “tonic” tail flagging outside the immediate presence of rattlesnakes (Hersek and Owings 1993), which generates statistical noise in playback studies. Elsewhere we have discussed evidence suggesting that tail flagging is a non-risky, ritualized behavior designed in part to deter pursuit by the snake (Swaigood et al. 1999a). Although tail flagging draws the snake’s attention, it does not appear to provoke striking (Towers and Coss 1990); thus, we do not consider it to be a costly behavior in terms of increasing risk of injury by snakes.
3. Percent time bipedal is the proportion of time the squirrel spent standing in bipedal posture during the 2-minute period following playback. This behavior is a useful measure of assessment and monitoring activities because bipedal posture is invariably associated with visual scanning and it affords the squirrel a better vantage to search for, identify and otherwise assess the snake. Presumably, this behavior entails no snake-related costs, as it almost always occurs well outside the snake’s strike range (personal observations).

To avoid type I errors associated with multiple tests on the three dependent variables, we analyzed these data with repeated-measures MANOVA, reporting the conservative Pillai’s Trace F -statistic (JMP version 3, SAS Institute, 1999). Proportion data were arcsine-transformed and frequency data were square root-transformed to correct for non-normality and heteroscedasticity. All data are reported as $\bar{x}$. Within-subject factors included trial and playback condition and the between-subject factor was reproductive category.

Following this analysis we used two sets of a priori planned comparisons, which do not require adjustments for family-wise error rates (Keppel 1991). In the first, maternal females were compared with nonmaternal females and males to test the hypothesis that maternal females would respond more strongly to rattling cues than males and nonmaternal females. For these tests we excluded data from control trials because we were interested in how reproductive categories differed during rattling trials only. Although not a planned comparison, we also report contrasts between males and nonmaternal females for comparison purposes. We constructed three additional planned comparisons to examine discrimination of playback sounds for each of the three reproductive categories: tones versus rattles; warm versus cold snake rattles; and large versus small snake rattles. If the overall F -statistic for the MANOVA model was significant, we analyzed each of the three dependent variables with univariate repeated-measures ANOVAs and planned comparisons to determine the relative importance of these behavioral variables in determining the discrimination response pattern.

Study 2: maternal responses to rattlesnakes and rattling sounds: the role of offspring age

In this study, we sought to determine whether risk-taking by maternal females increases with offspring age (as predicted by the offspring value hypothesis) or decreased with offspring age (as predicted by the offspring vulnerability hypothesis). We tested these hypotheses using both playbacks of rattling sounds and tethering of live snakes. In the playback study, we used data only in response to playbacks of the most dangerous snake, that is, the large warm condition, reasoning that assessment of maximum risk would produce the most profound effects. We used several of the same rattle playback trials described above; however, in some cases we did not know the age of the pups because we did not have observations of the day when pups first emerged from the burrow (at ca. 45 days of age: Linsdale 1946). However, we also obtained playback trials for some mothers for only some of the playback conditions, and therefore could not use them in the analyses for study 1. Thus we were able to supplement this dataset with new data, bringing the total sample size of playback trials of large warm rattles to females with pups of known age to $n=7$. These playbacks were conducted with females whose pups ranged in age from 21 days before to 21 days after first emergence from the natal burrow (ca. 24–66 days old).

In a similar manner, using a combination of previously reported and unreported data (Swaigood et al. 1999a), we were able to obtain eight trials with large tethered snakes. Pup age ranged from 0 to 21 days post-emergence. Pups of this age are becoming gradually more ambulatory, and thus it is possible that maternal response to snakes may change as a function of distance to pups, introducing a potential confound into analyses of offspring vulnerability and value hypotheses. However, all pups were below ground during the seven playback trials, so presence or proximity of young did not change systematically with pup age. For the tethered snake experiment, pups were above ground and within 3 m of the snake in three of four of trials conducted with pups 0–10 days of age and in three of four trials for pups 11–21 days of age, arguing against any confound between pup age and distance from mother during these trials. In addition, because snakes are ambush predators that wait at natal burrows (Hennessy and Owings 1988; Hersek 1990), it would behoove mothers to confront and monitor snakes near the natal burrow regardless of whether pups are currently present.

For each of these two studies, we selected two behavioral variables that should be most clearly associated with risk-taking. For tethered snakes we analyzed the amount of time the squirrel spent within 3 m of the snake, reasoning that the more time they spent in the vicinity, the more cumulative risk they are exposed to, especially since squirrels in the field often lose track of rattlesnakes (Hennessy and Owings 1988; Hersek and Owings 1993). The other measure, average distance to the snake while within this 3-m range,

is clearly related to potential risk (cf. Curio and Regelman 1985). For the same reason, we also used average distance to the playback speaker as a measure of risk-taking in the playback study. This measure perhaps reflects even greater risk because the squirrel is entering the domain of a highly dangerous snake whose location has not yet been verified visually. Finally, we also examined the duration of the reapproach to the speaker because squirrels that rush back in quickly are at greater risk of coming within strike range before determining the location of the snake whose rattle it just heard. We should point out that although in reality no snake is present, squirrels displaying bolder behavior are in fact being more assertive, and would be much more likely to engage the snake had one been present.

The methods for the playback study are described above. For details of the tethered snake study we refer the reader to our earlier paper (Swaigood et al. 1999a), but provide a brief summary here. The site, trapping and marking regime, identification of reproductive condition, and so forth are the same as for the playback study. We used four large rattlesnakes (562–1150 g) in the tethering trials, presenting one to each of the eight maternal squirrels. Approximately 1 m from the subject's natal burrow we drove a stake into the ground and attached the snake to a 40-cm monofilament line, allowing it to move freely but not escape. From a blind we recorded all behavior observed while the squirrel was within 3 m of the snake. The trial ended when the subject remained >3 m from the snake for >10 min. We performed simple regression of offspring age against log-transformed behaviors displayed by mothers.

Results

As originally reported in an earlier paper (Swaigood et al. 1999b), ground squirrel responses to rattling sounds were robust and sustained. After hearing rattle playbacks, ground squirrels adopted most of the responses typical of their interactions with live snakes. Most often, the squirrel backed away upon hearing the playback of rattling sounds, then stood bipedally and scanned the area before slowly reapproaching the speaker, pausing to tail flag as they reapproached.

Hypothesis 1: are maternal females more responsive to rattling playbacks than males and nonmaternal females?

Behavior among reproductive classes differed significantly (MANOVA: $F_{6,86}=2.2$, $P=0.047$). Planned comparisons showed that during rattling playback trials maternal females differed from nonmaternal females ($P=0.01$), but not males, though there is a nonsignificant trend ($P=0.07$). For purposes of contrast, we also compared males versus nonmaternal females, but as we predicted, this comparison did not approach significance ($P=0.28$). Univariate ANOVAs, while nonsignificant, suggest that tail-flagging accounts for much of these differences (males: 0.7 ± 0.9 ; mothers: 3.4 ± 0.8 ; nonmothers: 1 ± 0.8 ; $F_{2,16}=3.4$, $P=0.06$), whereas bipedal posture (males: 21.7 ± 6.1 ; mothers: 22.4 ± 6.4 ; nonmothers: 12.8 ± 6.3 ; $F_{2,16}=0.9$, $P=0.44$) and hesitancy to reapproach the playback speaker (males: 16.1 ± 8.4 ; mothers: 24.5 ± 7.2 ; nonmothers: 23.6 ± 7.2 ; $F_{2,16}=1.0$, $P=0.40$) differed little among males, maternal females and nonmaternal females.

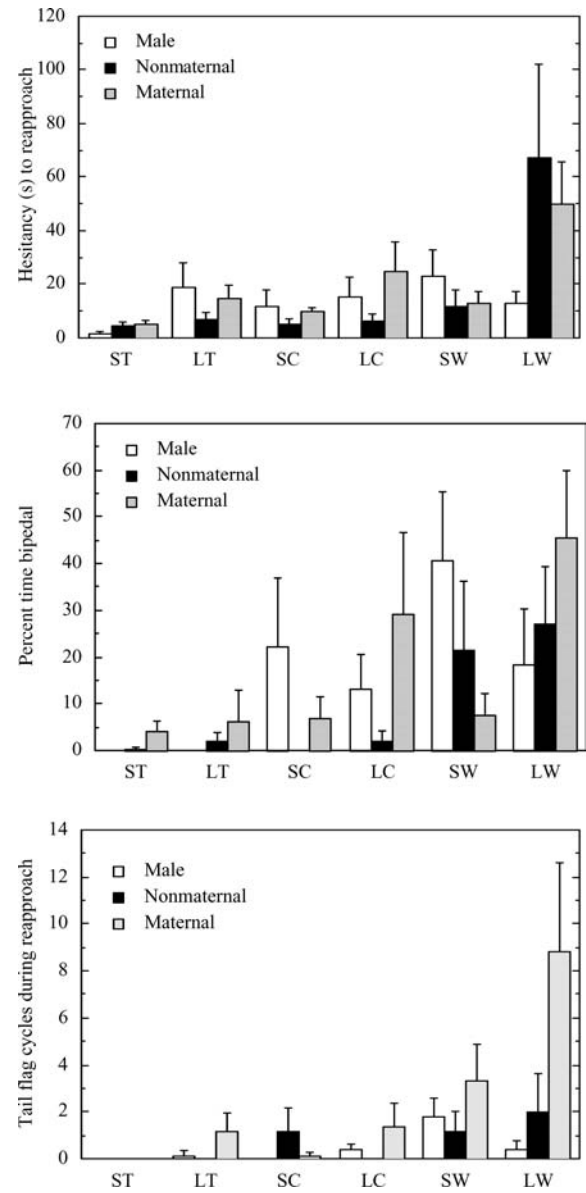


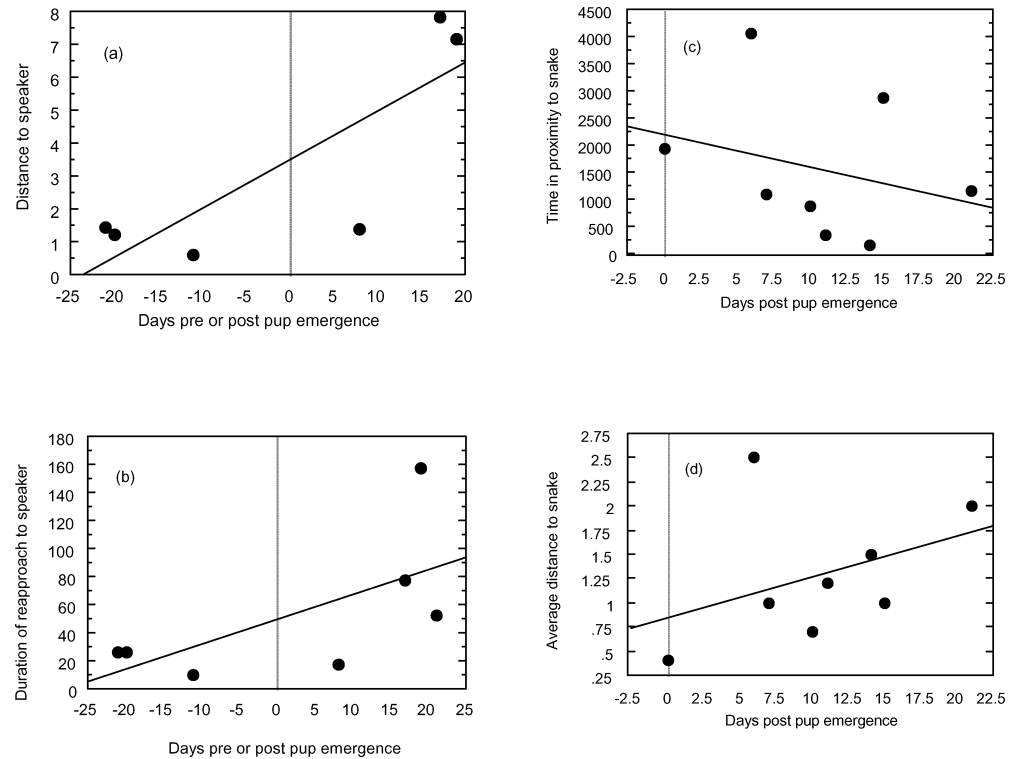
Fig. 1a–c Behavioral responses ($\bar{x} \pm SE$) to playbacks of rattling sounds to males, nonmaternal and maternal females. ST =soft tone; LT =loud tone; SC =small cold snake rattle; LC =large cold snake rattle; SW =small warm snake rattle; LW =large warm snake rattle. **a** Hesitancy (s) to reapproach the speaker following playback. **b** Percent time spent in bipedal posture. **c** Number of tail-flagging cycles during the reapproach to the playback speaker

Planned comparisons (including only data from rattling trials) revealed that these reproductive classes did not differ significantly with regard to hesitancy to reapproach the speaker or bipedal posture, but maternal females tail flagged significantly more than males ($P=0.003$) and nonmaternal females ($P=0.005$).

Table 1 Summary of results from planned comparisons for playback sounds examined separately for each reproductive class. Asterisk indicates significant tests ($P < 0.05$). In all cases, significant values are in the direction predicted, that is, heightened responses to rattles vs. tones, warm vs. cold snake rattling sounds, and large vs. small snake rattling sounds

Dependent variable	Tone versus rattle	Warm versus cold	Large versus small
	Male		
Hesitancy to reapproach	$P=0.22$	$P=0.74$	$P=0.83$
Bipedal posture	$P=0.004^*$	$P=0.23$	$P=0.27$
Tail-flag cycles	$P=0.28$	$P=0.31$	$P=0.68$
	Maternal female		
Hesitancy to reapproach	$P=0.0497^*$	$P=0.056$	$P=0.04^*$
Bipedal posture	$P=0.03^*$	$P=0.60$	$P=0.002^*$
Tail-flag cycles	$P=0.002^*$	$P<0.0001^*$	$P=0.04^*$
	Nonmaternal female		
Hesitancy to reapproach	$P=0.049^*$	$P=0.002^*$	$P=0.01^*$
Bipedal posture	$P=0.14$	$P=0.01^*$	$P=0.48$
Tail-flag cycles	$P=0.09$	$P=0.21$	$P=0.60$

Fig. 2a–d The effects of offspring age on maternal risk-taking in response to rattlesnakes and rattling sounds. **a** Average distance to the speaker following playback of rattling sounds from large warm rattlesnakes. **b** Time spent reapproaching the playback speaker after playback of rattling sounds from large warm rattlesnakes. **c** Time spent in proximity with (≤ 3 m) live tethered rattlesnakes. **d** Average distance to live tethered rattlesnakes while within 3 m of the snake



Hypothesis 2: are maternal females more sensitive to risk-related variation in playback sounds than males and nonmaternal females?

Behavioral discrimination among playback sounds differed significantly as a function of reproductive class (MANOVA reproductive class $\times$ playback interaction: $F_{30,135}=1.6$, $P=0.04$; Fig. 1). Mothers significantly discriminated among playback sounds for all three planned comparisons (tone vs. rattle: $P=0.004$; large vs. small: $P=0.0004$; warm vs. cold: $P=0.0001$). By contrast males only overtly discriminated tones from rattles ($P=0.009$) and nonmothers only differentiated cold from warm rattle playbacks ($P=0.01$).

Of the three behavioral measures, only tail-flagging activity yielded a significant reproductive class $\times$ playback interaction (ANOVA: $F_{10,48}=2.6$, $P=0.01$). Planned

comparisons show that mothers strongly differentiate playback sounds with their tail-flagging activity (Table 1). Mothers tail flagged more to rattles than tones, warm snake rattles than cold snake rattles, and large snake rattles than small snake rattles. By contrast, males and nonmothers did not discriminate among playback sounds on the basis of their tail-flagging activity. These differences in discrimination patterns among reproductive classes were less pronounced for the behavioral variables “hesitancy to reapproach the playback speaker” and “bipedal posture.” Mothers were more hesitant to reapproach the speaker in response to (1) rattles than tones, (2) warm snake rattles than cold snake rattles (albeit not quite significant: $P=0.056$), and (3) large snake rattles than small snake rattles. Nonmother planned comparisons showed the same pattern, but all three were significant, whereas males took the same amount of time to

reapproach the playback speaker regardless of the nature of the playback sound. Mothers spent more time standing bipedally in response to rattles than tones and large snake rattles than cold snake rattles. Nonmothers stood bipedally more following playback of warm snake rattles than cold snake rattles, while males stood bipedally more after rattles than tones. In sum, of the nine planned comparisons, mothers attained statistical significance for seven (and one marginally nonsignificant), nonmothers for four, and males for only one.

Hypothesis 3: does antisnake risk-taking among maternal females increase or decrease as offspring age?

If maternal responses to rattlesnakes and rattling sounds are governed by offspring value (increasing with increasing offspring age), then mothers should invest more in defense as their pups grow older. However, in response to playbacks of rattling sounds from large warm rattlesnakes maternal female ground squirrels remained significantly farther away from the playback speaker with increasing offspring age (simple regression: $n=7$; $r^2=0.67$; $P=0.047$; Fig. 2a), suggesting that mothers are less inclined to engage in risky behavior when they have older pups. With older offspring they also progressed somewhat more slowly while reapproaching the playback speaker, an affect that was not significant ($n=7$; $r^2=0.40$; $P=0.13$; Fig. 2b). The relationships between pup age and their mothers' antisnake behavior in response to live snakes, while nonsignificant, were also inconsistent with the offspring value hypothesis. Time spent in proximity (3 m) to the snake and average distance to the snake while in proximity were not associated with pup age ($n=8$; $r^2=0.08$; $P=0.49$; Fig. 2c; and $r^2=0.15$; $P=0.34$; Fig. 2d, respectively). Indeed weak trends evident in Fig. 2c and d suggest the opposite: if anything, as offspring grow older mothers remain farther away from the tethered snake and spend less time in proximity with the snake, where presumably they could better monitor or dissuade the snake. These data suggest that with larger sample sizes results would show either no association between maternal risk-taking and offspring age or perhaps decreased risk-taking.

Discussion

How does sex and parity influence responses to rattling sounds?

Reproductive classes differed significantly in the MANOVA model, with mothers differing significantly from nonmothers and marginally nonsignificantly from males, providing support for the hypothesis that mothers should be more responsive to acoustic cues from rattlesnakes. This effect was driven largely by maternal female tail flagging. Mothers tail flagged significantly more than males and nonmothers. Tail flagging is a conspicuous

activity well designed for signal function. Maternal tail flagging does not necessarily indicate that this signal is directed toward pups. It is common before pup emergence (Hersek and Owings 1993), and was common in the playback trials, when pups were rarely above ground. Tail flagging might recruit adult squirrels to mob the snake or become more vigilant "spotters" (Hersek and Owings 1993). The snake might also be a target of tail flagging, which may amplify the effects of other snake-directed activities such as close approach and substrate throwing, and serve a pursuit deterrent function (*sensu* Hasson 1991). These activities may encourage the snake to leave by advertising the costs associated with the prey's (or its mother's) efforts to monitor and perhaps harass the snake (Swaigood et al. 1999a). Rattlesnakes are highly cryptic sit-and-wait ambush predators, and allowing one to remain in the area—or losing track of its location—could and does prove fatal to pups (personal observations). In contrast, adults risk injury, not death, if they stumble onto a rattlesnake, which—because adults are not prey—would only strike an adult if threatened.

Consistent with the general lack of paternal care in California ground squirrels, male responses to rattling playbacks seem related to self preservation: they stood bipedally and engaged in cautious assessment, but signaled less in comparison with mothers. In a previous study, we were unable to find any evidence of paternal defense of young (Swaigood et al. 1999a). Such lack of paternal care may reflect the high level of paternity uncertainty in the species (Boellstorff et al. 1994) or, alternatively, because mammalian mothers have greater opportunity for parental care because of their association with offspring at birth (Trivers 1972). The former hypothesis is supported by comparison with black-tailed prairie dogs, where harem-defending males have greater paternity certainty and invest more time and energy in antisnake behavior than do mothers (Loughry 1987; Loughry 1993).

Reproductive classes also differed markedly in their overt behavioral differentiation between the playback sounds, as suggested by a significant playback $\times$ reproductive class interaction in the MANOVA. Planned comparisons for each of the three behaviors, when significant, were always in the direction predicted, that is, heightened responses to acoustic cues from more dangerous snakes. Males displayed little evidence of discrimination, only differentiating tones from rattles on the basis of bipedal posture. Nonmothers were more discriminating: they were slower to reapproach the playback speaker following playback of rattles versus tones, warm versus cold snake rattles, and large versus small snake rattles, and stood bipedally longer in response to warm versus cold snake rattles. Mothers were the most discriminating. Of the nine planned comparisons for mothers, eight were significant or marginally nonsignificant, and they were the only reproductive class to differentiate between playback sounds with their tail-flagging activity. Mothers' tail flagging was most dramatically elevated following rattling sounds from

large warm snakes, the most dangerous and most likely snake to be hunting pups in the colony. Why is it so important for mothers to track snake dangerousness with tail-flag signals? Perhaps because tail-flagging reflects not only differential risk posed to the mother, but also the different threats posed by large versus small and warm versus cold snakes to her offspring. If tail flagging dissuades the snake or recruits other squirrels to help monitor or expel the snake, then increased tail flagging may amplify this function. For example, under the pursuit-deterrence hypothesis, increased signaling intensity advertises the signaler's condition and ability to escape or defend themselves, and should more effectively deter the predator (Woodland et al. 1980; Hasson 1991). Risks taken during snake harassment appear to be constrained by snake dangerousness (Swaigood et al. 1999a), and tail flagging may be a less risky alternative to escalated aggression.

Clearly, California ground squirrels possess sophisticated mechanisms for assessing risk posed by their most significant adversary, the northern Pacific rattlesnake. Squirrels approach and harass snakes, provoking the snake to rattle and then extract acoustic cues related to at least two dimensions of snake dangerousness: size and temperature (Swaigood et al. 1999a, 1999b). In their use of acoustic cues of size, squirrels exploit the same relationship between pitch and body size that animals use in intraspecific combat (Davies and Halliday 1978). These assessment strategies, while important to all ground squirrels, are used to a greater degree by maternal females that need to defend their young.

How does offspring age influence antisnake risk-taking by maternal females?

Models of parental defense (Andersson et al. 1980; Montgomerie and Weatherhead 1988) and parental care in general (Clutton-Brock 1991) predict that parents should pay more costs to increase offspring survival as offspring age increases. This is because the probability of offspring survival to adulthood increases with age and therefore older offspring are more valuable. However, as offspring age they also become less vulnerable and benefit less from parental assistance; thus, parental defense is predicted to decrease with offspring age. Our results for maternal ground squirrel responses to tethered snakes and large warm snake rattling sounds are clearly inconsistent with the offspring value hypothesis. All measures of risk-taking decreased somewhat as offspring grew older; average distance to the speaker increased significantly with offspring age, indicating less risk-taking by the mother with older offspring. We conclude that maternal females' antisnake behavior is not influenced by offspring value or that any such influence is offset by decreasing offspring vulnerability. Based on these results, we do not reject the offspring vulnerability hypothesis and tentatively accept it subject to further confirmation.

The developmental stages covered by the analyses of offspring age presented here run from about 3 weeks before to 3 weeks after pup emergence for the playbacks and cover the 3 weeks following pup emergence for the tethered snake presentations. Rattlesnakes predate on squirrel pups throughout this period (above and below ground), but evidence suggests that pups are becoming less vulnerable to rattlesnakes, especially during the latter part of the post-emergence period. Weight gain affords pups some protection through increased capacity to neutralize snake venom, and increased strength, agility and behavioral competence enhance their ability to execute the dramatic evasive leaps characteristic of squirrels when struck at by snakes (Poran and Coss 1990). Fitch (1949) noted that rattlesnakes focus predation effort on newly emerged pups and Hennessy and Owings (1988) reported that most snake activity at squirrel colonies occurs during approximately 2 weeks before and after pup emergence. Rattlesnake "interest" in pups declines rapidly thereafter and so do signs of maternal defense.

Similar results have been found for maternal mice defending their pups against infanticidal conspecifics (Wolff 1985). Attacks on pups decrease rapidly after pups reach approximately 18 days of age, when pups are large enough to deter attackers. Mothers are extremely aggressive toward conspecific intruders and are successful at preventing infanticide. Such maternal attacks decrease rapidly at the time pups are reaching this less vulnerable stage (Svare 1981). We suspect that if more researchers use mammalian species to test predictions deriving from theoretical models of parental defense, we will find that offspring vulnerability often plays a larger role in mammals than in birds. Most studies of parental defense in birds are conducted with altricial species where young have little chance of evading or defending themselves against predators before fledging. Thus, vulnerability remains high throughout nesting and declines precipitously at fledging, at which time a dramatic decline in parental defense is observed. This stands in sharp contrast to many mammals, where young gain muscle mass, weight, speed and agility more gradually, punctuated by occasional maturational "leaps."

The role of changing offspring vulnerability in parental defense gets more complex, even for altricial birds, when several predators are considered. For example, in both magpies and white-crowned sparrows parental defense follows different patterns of change with different predators as young mature, with peaks in defense coinciding with the time of greatest offspring vulnerability to the particular predator (Patterson et al. 1980; Buitron 1983; see also, Hauser 1988). Thus, offspring vulnerability and parental defense tactics may not change monotonically with development of the young, but may track predator-specific patterns of changing threat.

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