Available online at www.sciencedirect.com

ScienceDirect

# Snake scent application in ground squirrels, *Spermophilus* spp.: a novel form of antipredator behaviour?

BARBARA CLUCAS*, MATTHEW P. ROWE†, DONALD H. OWINGS*‡ & PATRICIA C. ARROWOOD§

*Animal Behavior Graduate Group, University of California, Davis
†Department of Biological Sciences, Sam Houston State University
‡Department of Psychology, University of California, Davis
§Department of Fishery and Wildlife Science, New Mexico State University, Las Cruces

Chemical substances produced by one species are sometimes found on the body of another species. Animals often ingest such foreign substances and sequester them into their integument, but here we report a case of direct application of heterospecific substances to the body. California ground squirrels, *Spermophilus beecheyi*, and rock squirrels, *Spermophilus variegatus*, apply scent derived from their major predator, rattlesnakes, *Crotalus* spp., by chewing shed rattlesnake skins and licking their fur. We found that the sequence of body areas licked during application was essentially the same for the two species. We consider three hypotheses regarding the function of this 'snake scent application' (SSA): antipredator defence, ectoparasite defence, and conspecific deterrence. To test these hypotheses, we assessed patterns of species and sex/age class differences in application quantity and compared them with patterns reflecting differences in the importance of predation, flea loads and conspecific aggression as sources of selection. We found no species differences in application quantity; however, juveniles and adult females of both species engaged in longer bouts of application than adult males. This pattern of sex/age class differences in SSA supports only the antipredator hypothesis because juveniles are most vulnerable to predation and adult females actively protect their young. We found no evidence to support either the ectoparasite defence or conspecific deterrence hypotheses. Thus, SSA behaviour may be a novel form of chemical defence against predation.

© 2007 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Animals opportunistically use resources in their environment for novel purposes, such as tools or material for nest construction (Lestel & Grundmann 1999). In some cases, the commandeered substances are produced by other animal species (e.g. Williams et al. 2004). Certain amphibian and avian species, for example, acquire chemicals from ingested prey and sequester them into their integument

*Correspondence: B. Clucas, Animal Behavior Graduate Group, One Shields Avenue, UC Davis (Young Hall), University of California, Davis, CA 95616-8686, U.S.A. (email: baclucas@ucdavis.edu). D. H. Owings, Department of Psychology, One Shields Avenue, University of California, Davis, Davis, CA 95616-8686, U.S.A. M. P. Rowe, Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341, U.S.A. P. C. Arrowood, Department of Fishery and Wildlife Science, New Mexico State University, Las Cruces, NM 88003-8003, U.S.A.*

(Daly 1997; Bartram & Boland 2001). Other animals, representing a wide array of taxa, directly apply foreign substances onto their integument, an activity called 'self-application' or 'anointment' (Weldon 2004; see Table 1).

Chemicals sequestered internally by animals are typically thought to reduce the animals' palatability to predators. Consistent with this hypothesis, animals that sequester toxic chemicals are often aposematic (e.g. Dumbacher & Fleischer 2001). In contrast, many species that directly apply substances to their skin lack conspicuous coloration and use substances that are odiferous rather than toxic (see Table 1). Thus, self-applied chemicals might be used by the applier in different ways than chemicals sequestered internally. Indeed, several studies have proposed that odorous applied substances repel predators and/or ectoparasites (Kobayashi & Watanabe 1986; Xu et al. 1995; Weldon et al. 2003; Weldon 2004; Carroll et al. 2005) or

**Table 1.** Examples of applied substances and proposed functions

| Proposed function | Substance source | Applier | Application behaviour |
| --- | --- | --- | --- |
| Antipredator | Snake | Chipmunks[1] | Chew on snake carcass and apply by licking into fur |
| Antipredator | Weasel | Rats[2] | Chew filter paper saturated with weasel anal gland secretions and apply by licking into fur |
| Antipredator (social) | Toad | Hedgehogs[3] | Chew toad skin then apply by licking into fur |
| Antipredator | Brown algae | Decorator crabs[4] | Cover carapace with algae |
| Ectoparasite defence | Ants | Birds[5] | Sit on ant mound and/or bite ant(s) and apply to feathers with beak |
| Ectoparasite defence | Millipedes | Birds[6] | Bite millipede and apply to feathers with beak |
| Ectoparasite defence | Millipedes | Primates[7] | Bite millipede and rub secretions into fur |
| Ectoparasite defence | Leaves | Primates[8] | Rub chewed leaves into fur |
| Ectoparasite defence | Catnip | Felines[9] | Roll on catnip |
| Ectoparasite defence | Tree resin | Coatis[10] | Dig claws into resin seeping from tree and apply to fur |
| Social | Carcasses | Hyaenas[11] Wolves[12] | Roll on prey carcass |
| Integration into an ant colony | Ants | Beetles[13] | Cover carapace with dead ants |
| None given | Ants | Grey squirrels[14] | Roll around on ants and/or ant hills |

1. Kobayashi & Watanabe 1986; 2. Xu et al. 1995; 3. Brodie 1977, D'Have et al. 2005; 4. Stachowicz & Hay 1999; 5. Clark 1990, Fauth et al. 1991, Husak & Husak 1997, Rodgers et al. 1998, Osborn, 1998, Milton & Dean 1999, Craig 1999, Gwinner et al. 2000; 6. Harrup 1992, Parkes et al. 2003; 7. Birkinshaw 1999, Valderrama et al. 2000, Zito et al. 2003, Weldon et al. 2003; 8. Baker 1996, Campbell 2000, Zito et al. 2003; 9. Tucker & Tucker 1988, Bernier et al. 2005; 10. Gompper & Holyman 1993; 11. Drea et al. 2002; 12. Zimen 1981; 13. Vandermeer & Wojcik 1982; 14. Bagg 1952, Hauser 1964.

affect the behaviour of conspecifics (Kobayashi 2000; Drea et al. 2002; D'Have et al. 2005).

Several rodent species apply substances acquired from their snake predators by chewing the source (e.g. shed skins, carcasses) and licking their fur. This behaviour, termed 'snake scent application' (SSA), was first reported in Siberian chipmunks, *Eutamias sibericus asiaticus* (Kobayashi & Watanabe 1986) and was later observed in some ground squirrels, *Spermophilus* spp. (Owings et al. 2001) and grasshopper mice, *Onychomys torridus* (M. Rowe, unpublished data). Similarly, rice-field rats, *Rattus rattoides*, chew on and apply the anal gland secretions of weasels, *Mustela sibirica*, a rodent predator, (Xu et al. 1995). This behaviour in these rodent species appears similar to the phylogenetically conserved head-to-tail (cephalocaudal) grooming sequence (see Berridge 1990), suggesting an evolutionary derivation from this grooming sequence. Here we evaluate the form of SSA in two ground squirrel species by presenting California ground squirrels, *S. beecheyi*, and rock squirrels, *S. variegatus*, with shed skins from local rattlesnake species, *Crotalus* spp. (Fig. 1) and quantifying the sequence and location of application to different body areas.

Predator scent application has been proposed to serve an antipredator function (Kobayashi & Watanabe 1986; Xu et al. 1995), but alternative explanations are also plausible. We consider three functional hypotheses of SSA in ground squirrels: antipredator defence, ectoparasite defence, and conspecific deterrence. We test these hypotheses by comparing patterns of species and sex/age class differences in amount of application with patterns reflecting differences in importance of predators, flea loads and conspecific aggression. Contrasting patterns of selection from these three sources both between the two species and among different age and sex classes of squirrels can provide insights into the function of SSA.

Both of the closely related (Herron et al. 2004) but geographically separate ground squirrel species studied here have been subjected to rattlesnake predation for many millennia (Coss 1999), which has led to the evolution of a complex defence system that includes venom resistance in adults and sophisticated antisnake behaviour (Owings & Coss 1977; Rowe & Owings 1978; Hennessy & Owings 1988; Biardi 2000; Owings et al. 2001). However, California ground squirrels live at higher densities than rock squirrels (compare Fitch 1948 with Shriner & Stacey 1991) and show greater sexual differentiation in size (Owings et al. 2001) and apparently aggressiveness. In addition to age/sex class differences in the impact of predators, ectoparasites and conspecifics (Fitch & Twining 1946; Owings et al. 1979; Bursten et al. 1997), we use these species differences to make predictions for each functional hypothesis.

## Antipredator

SSA might alter ground squirrel odour and thereby either reduce detectability to predators or repel other rattlesnakes motivated to avoid hunting in the same area as a conspecific. Juvenile ground squirrels are the most susceptible to predation, especially from rattlesnakes because their small size limits the volume of venom they can neutralize, and because they are less likely to evade predators (Fitch & Twining 1946; Owings & Coss 1977; Poran et al. 1987; Mateo, 2007). Nevertheless, adult females actively protect their offspring from rattlesnakes (e.g. Swaisgood et al. 2003), share burrows with vulnerable related juveniles (Johnson 1981; Boellstorff & Owings 1995), and generally deal more directly with predators than do adult males (e.g. through alarm calling; Dunford 1977; Sherman 1977; Schwagmeyer 1981). Therefore, we predicted that juveniles and adult females would SSA more than adult males in both species if it serves an antipredator function.

(a) California ground squirrel



(b) Rock squirrel



Chewing rattlesnake shed                    SSA to flank

**Figure 1.** Application of snake scent in ground squirrels. (a) California ground squirrel chewing on shed skin of northern Pacific rattlesnake and applying scent to flank by licking fur. (b) Rock squirrel chewing on shed skin of western diamondback rattlesnake and applying scent to flank by licking fur. Arrows indicate rattlesnake shed skin.

## Ectoparasite Defence

Ground squirrels may apply snake scent to reduce their ectoparasite load either by masking host odour cues or by repelling parasites (e.g. Clark & Mason 1988; Hemmes et al. 2002; Weldon et al. 2003). Thus, for this hypothesis we predicted that SSA duration would correlate positively with an individual's flea load. Juvenile California ground squirrels have more fleas than do adults (Bursten et al. 1997), and should therefore engage in more SSA than adults. We measured flea loads in both species to test potential correlations with SSA duration and to confirm age differences in flea loads.

## Conspecific Deterrence

SSA might provide the scented ground squirrel with a competitive advantage by distracting a conspecific adversary during an aggressive interaction (e.g. Ropartz 1968). We predicted that adult males should SSA more than both adult females and juveniles because males have been shown to engage in more aggressive interactions in California ground squirrels (Owings et al. 1979; Owings & Leger 1980). This greater aggressiveness by males than females is associated with male-biased sexual size differences in this species (Owings et al 2001). We further predicted that sex differences in SSA would be more pronounced in California ground squirrels than rock squirrels, because rock squirrels show smaller sex differences than California ground squirrels with regard to both size and conspecific tolerance (Owings et al. 2001). We tested this hypothesis by measuring levels of aggressive interactions during natural observations of both California ground squirrels and rock squirrels and comparing them with SSA duration.

## METHODS

## Study Species

California ground squirrel trials were conducted at Lake Solano County Park (38°29′N, 122°1′W; hereafter Solano) west of Winters, California (June−August 2004) and rock squirrel trials at Caballo Lake State Park (32°58′N, 107°18′W; hereafter Caballo) north of Caballo, New Mexico (August 2002 and April 2004). Rattlesnakes are found at both sites: northern Pacific rattlesnakes, *Crotalus viridis oreganus*, at Solano, and western diamondbacks, *Crotalus atrox*, at Caballo. Before testing, squirrels were trapped using Tomahawk traps baited with black sunflower seeds. Individuals were weighed, anaesthetized with ketamine hydrochloride, Passive Integrated Transponder (PIT)-tagged and individually marked with black Nyanzol dye. We determined sex by external genitalia and age by both evidence of prior enlargement of scrotum or teats and weight (individuals under

400 g were typically juveniles). In 2004 trials, we obtained an estimate of flea loads by holding anaesthetized squirrels on their back and scanning the ventrum from head to tail, counting fleas for 1 min. Once the anaesthetic had fully worn off, animals were released at the site of capture.

### SSA Trials

We attempted to test an equal number of individually marked squirrels for each age/sex class (adult females, adult males, and juveniles). Testing sites were established by laying out bait approximately 3 m from an active burrow 1−2 h before starting a trial. A shed skin of the local rattlesnake species was then staked out using fishing line tied around the skin and hooked to a 13-cm stake that was driven into the ground. The stimulus was surrounded by sufficient bait to attract squirrels but not enough to elicit extended bouts of feeding (8−10 pieces). Shed skins came from different rattlesnakes for each trial, and were stored frozen until use (to preserve scent), always handled with latex gloves, and used for only one trial. Each stake site was used once and sites were approximately 20−500 m apart. We made observations from a parked vehicle 20−25 m from the stimulus (squirrels at both locations were accustomed to vehicles).

Trials began when a marked individual came within 50 cm of the shed skin and lasted for 30 min. Squirrels were recorded on video during trials using a digital camcorder, and instantaneous time samples were narrated onto the audio track of the videotape every 30 s, noting the focal squirrel's distance from the skin and main activity (i.e. chewing snake skin and applying scent, moving, foraging, social, or out of sight).

### Data Collection and Analysis

Frame-by-frame video analysis of SSA was conducted for every marked squirrel that showed the behaviour. The total number of licks to each body location and the sequence of body areas that received application (see Fig. 2 and Supplementary Material, Fig. S1) were scored using an event recorder (JWatcher 1.0, Animal Behaviour Laboratory, Macquarie University, Sydney, Australia; Blumstein et al. 2006). The proportion of licks to the body area/total licks was calculated for each individual and compared across species and age classes (unequal samples sizes did not allow for sex comparisons). Because of non-normal distributions, we used nonparametric statistics to analyze potential differences (Mann−Whitney $U$ test) and used a Bonferroni corrected critical value to correct for multiple tests. Discrete-time sequential analysis was performed to quantify frequency and transition probabilities between body areas anointed (with accompanying z scores and $P$ values; JWatcher 1.0; Blumstein et al. 2006).

Next, SSA trials were scored from video in real-time to measure the total amount of time spent applying scent during trials (SSA duration, in s). In addition, we calculated the proportion of time samples during which scent application occurred by dividing the number of samples that included scent application by the total time samples that subjects spent within 1 m of the shed skin (hereafter SSA/1 m). This adjusted for the different amounts of time that individuals had access to the stimuli; for example, some squirrels spent much of their time near the skin engaged in SSA, but scored only low SSA durations because they were supplanted by others from the shed skin after only a brief period of access. Initial analyses of the two dependent variables of SSA quantity across the factors species and age/sex (adult males, adult females, and juveniles) showed that adult males of both species had many zeros and their distributions were not normal. Therefore, we compared across species and age/sex classes using a nonparametric statistic (Mann−Whitney $U$ test using a Bonferroni corrected critical value to correct for multiple tests).

Flea loads were scored by the number seen during a 1-min count and ordinally categorized as: none (0), low (1−2), medium (3−4), and high (5+) (observed range was 0 to 10). By limiting our search to 1 min, we avoided recounting fleas as we scanned the squirrels' ventrums. We first tested for species, sex and age differences in flea load using a multinomial logistic regression. We then performed a linear regression to test for a relationship between flea load and the dependent variable SSA duration. Because sample sizes were not equally distributed across flea load categories and our data were ordinal, we used a nonparametric test (Spearman rank correlation).

Before conducting any SSA trials in the corresponding season, we measured number of aggressive interactions across age/sex classes during natural observations of rock squirrels at Caballo and California ground squirrels at Solano (July−August 2004). Squirrels were observed for 20 min and the numbers of aggressive interactions were scored continuously. An interaction was aggressive if the focal squirrel actively supplanted another squirrel (chased, pushed, bit). All age and sex classes were scored in California ground squirrels (adult males = 7, adult females = 13, juveniles = 10), whereas only adults were available for scoring in rock squirrels (adult males = 10, adult females = 21). Levels of aggressive interactions were then compared between species and across sex and age classes (Mann−Whitney $U$ test).

All statistical tests were performed in SPSS 11.0.2 (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

### Form of SSA

Trial videos for 17 of 30 California ground squirrels (11 adults and 6 juveniles), and 29 of 41 rock squirrels (21 adults and 8 juveniles) were sufficiently clear for detailed analysis of form. The sequential pattern of SSA involved stereotyped progression typically through the following phases: chewing the shed skin, twisting to the side and licking the flanks, grabbing the tail with forepaws and then licking along the length of the tail from base to tip (Supplementary Material, Fig. S1). Such sequences were often repeated, and squirrels also occasionally progressed from flanks to hindlegs and genital area rather than to the tail (Supplementary Material, Fig. S1).
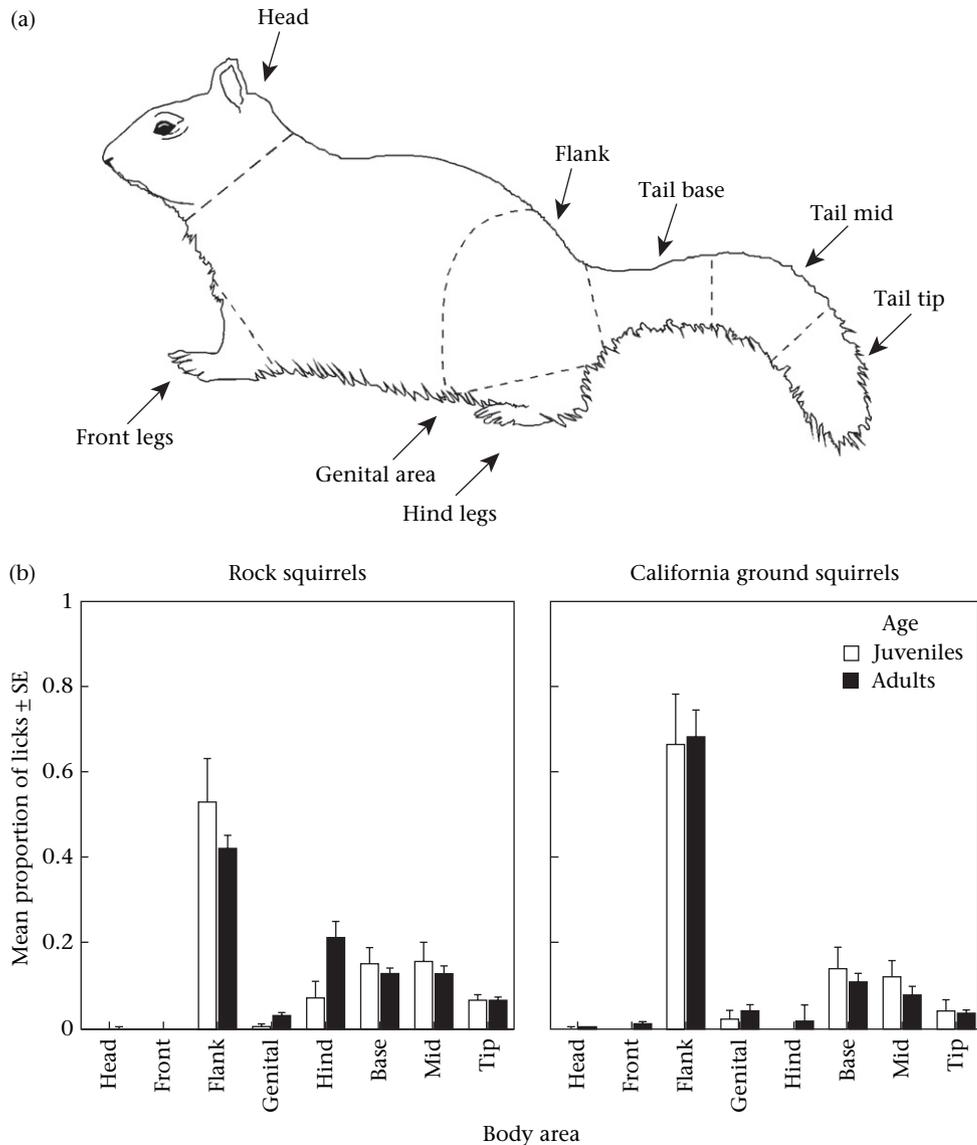
**Figure 2.** (a) Areas of the body applied with snake scent. (b)Mean +SE proportion of licks to each body area for adults and juveniles of both California ground squirrels and rock squirrels.

Both species applied the most snake scent to their flanks and tail, focused secondarily on their rear legs and occasionally applied scent to their genital area, front legs and head (Fig. 2). Rock squirrels licked their flanks less and hindlegs more than the California ground squirrels (Mann−Whitney $U$ test: flank: $U = 120.5$, $P = 0.004$; hindleg: $U = 60.5$, $P < 0.001$; Fig. 2b), but these species differences were generated by rock squirrel adults, and not juveniles. Rock squirrel adults differed from California ground squirrel adults (flank: $U = 44.0$, $P = 0.005$; hindleg: $U = 15.5$, $P < 0.0001$) and California ground squirrel juveniles (hindleg: $U = 3.0$, $P < 0.0001$), whereas rock squirrel juveniles did not differ from either California ground squirrel adults (flank: $U = 26.0$, $P = 0.137$; hindleg: $U = 30.0$, $P = 0.182$) or juveniles (flank: $U = 17.0$, $P = 0.361$; hindleg: $U = 12.0$, $P = 0.052$; all tests were performed using a Bonferroni critical value of 0.0125; Fig. 2b). Within both species,

however, there were no age differences in proportion of licks to any body area (Fig. 2b).

## Function of SSA

Trials on 30 California ground squirrels (14 adult females, 9 adult males, and 7 juveniles) and 41 rock squirrels (16 adult females, 14 adult males, and 11 juveniles) were used to calculate SSA quantity measures (SSA duration and SSA/1 m).

### Antipredator

The data supported only the antipredator hypothesis. This hypothesis predicted that juveniles and adult females of both species should engage in more SSA than adult males, and that the two species should show comparable
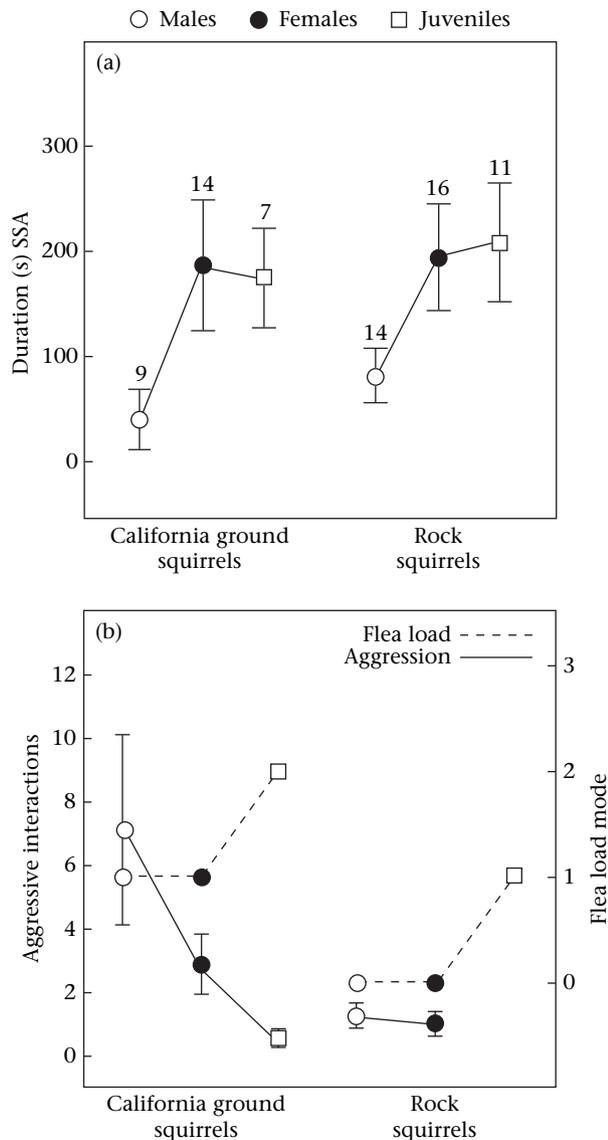
**Figure 3.** (a) Mean ± SE duration of SSA in s (numbers above represent sample sizes) and (b) Mean ± SE number of aggressive interactions and flea load modes in California ground squirrels and rock squirrels.

amounts of SSA. The results were consistent with these predictions (Fig. 3a). The quantity of SSA did not differ significantly across species within age/sex classes for either dependent variable (Mann−Whitney $U$ test: SSA duration: adult males $U = 54.5$, $P = 0.332$, adult females $U = 104.0$, $P = 0.550$, juveniles $U = 27.5$, $P = 0.792$; SSA/1 m: adult males $U = 54.0$, $P = 0.317$, adult females $U = 79.0$, $P = 0.110$, juveniles $U = 14.5$, $P = 0.093$). However, both adult females and juveniles engaged in more SSA than adult males (Mann−Whitney $U$ test: SSA duration: adult males versus adult females $U = 191.0$, $P = 0.005$, adult males versus juveniles $U = 94.0$, $P = 0.002$; SSA/1 m: adult males versus adult females $U = 202.0$, $P = 0.009$, adult males versus juveniles $U = 105.0$, $P = 0.006$; all tests were performed using a Bonferroni corrected critical value of 0.025).

## Ectoparasite defence

We found no support for the ectoparasite defence hypothesis. This hypothesis predicted that juveniles of both species should engage in more SSA than adults because juveniles carry heavier flea loads. Juveniles of both species did in fact carry heavier flea loads (multinomial logistic regression: $N = 107$; age: $\chi^2_3 = 9.397$, $P < 0.05$; Fig. 3b). But the pattern of age differences in SSA was not consistent with this prediction. Although juveniles engaged in more SSA than adult males, they were equivalent to adult females (Fig. 3a). Furthermore, California ground squirrels had greater flea loads than rock squirrels (same multinomial logistic regression as above: $N = 107$; species: $\chi^2_3 = 30.932$, $P < 0.0001$; Fig. 3b), but did not engage in more SSA than rock squirrels (Fig. 3a). Finally, individual flea load was not significantly correlated with individual SSA duration (Spearman rank correlation: $r_S = -0.033$, $N = 45$, $P = 0.829$).

## Conspecific deterrence

We found no support for the conspecific deterrence hypothesis. This hypothesis predicted that more aggressive adult males should engage in more SSA than juveniles and adult females, and that these differences should be more pronounced in California ground squirrels. In California ground squirrels, adult males were more aggressive than juveniles (Mann−Whitney $U$ test: $U = 9.5$, $P = 0.008$; Fig. 3b) and tended to be more aggressive than adult females ($U = 24$, $P = 0.081$; Fig. 3b). But this contrasted sharply with their differences in SSA (compare Fig. 3a with Fig. 3b), where males engaged in significantly less SSA than both females and juveniles. As expected, adult rock squirrels showed less pronounced sex differences in aggression (in fact, no sex differences; $U = 85$, $P = 0.362$; Fig. 3b), but males engaged in significantly less SSA than females (and juveniles; Fig. 3a). Finally, adult rock squirrels were significantly less aggressive than adult California ground squirrels ($U = 187.5$, $P = 0.013$; Fig. 3b), but these species did not differ in amount of SSA (Fig. 3a). Although the species difference in adult aggression was driven primarily by males (adult males: $U = 15$, $P = 0.047$; adult females: $U = 93$, $P = 0.098$; Fig. 3b), neither sex showed species differences in SSA (Fig. 3a).

## DISCUSSION

### Form of SSA in Ground Squirrels

California ground squirrels and rock squirrels applied rattlesnake scent in a similar stereotyped sequence, typically beginning with the flank and progressing to the tail tip. Form differed only in the proportion of licks to a few body areas, and these species differences were more pronounced for adults than for juveniles, suggesting that they are generated by divergent developmental trajectories of SSA form (cf. Baier et al. 2006). These findings provide a basis for the working hypothesis that SSA is homologous in these two ground squirrel species.

Self-application with foreign substances is found in many animal taxa (Table 1). In particular, rodents (e.g.

chipmunks, rats, and mice) apply substances produced by their predators (Kobayashi & Watanabe 1986; Xu et al. 1995; M. Rowe, unpublished data). The form of this application behaviour across rodent species is very similar to that reported here for ground squirrels and also to the stereotyped cephalocaudal grooming pattern conserved across this group as a whole (see Berridge 1990). This similarity across distantly related rodent taxa suggests that predator scent application is derived from this evolutionarily old grooming pattern (see Tinbergen 1952). Such novel use of the cephalocaudal grooming pattern in a new functional context has a precedent: California ground squirrels also use it as an agonistic social display (Bursten et al. 2000), as do rock squirrels (unpublished observations). However, if this hypothetical evolutionary scenario is correct, then the transition to the extant state of SSA has involved deletion of the face and head rubs that typically precede posterior licking in cephalocaudal grooming.

## Function of SSA in Ground Squirrels

The form of application may have implications for the function of the behaviour. Applying scent to the tail and posterior of the body may facilitate olfactory masking because anal glands are a major source of odour (Salmon & Marsh 1989). Alternatively or in addition, application to the tail may enhance dissemination of the scent via movement of the tail or piloerection of the tail fur. However, the pattern of variation in the quantity of SSA with species and age/sex classes offer particular insight into function.

We found that adult females and juveniles spent more time applying scent than did adult males in both California ground squirrels and rock squirrels with no differences between species. These results match the predictions of the antipredator hypothesis. Predation is more important for adult females and juveniles in both these species because mothers actively protect their vulnerable young from rattlesnakes and other predators. These closely related species have previously been shown to share several antipredator behavioural and physiological mechanisms (Owings et al. 2001; Biardi 2000).

The incidence of predator scent application has not been found to differ between sex or age classes in rats and chipmunks (Xu et al. 1995; Kobayashi & Watanabe 1986), but may yet prove to differ with the use of more sensitive measures involving durations of application behaviour. Juvenile hedgehogs applied substances more than adult males, a finding similar to our results, but in contrast to our results, adult males applied more than adult females (D'Have et al. 2005). Such interspecies variation in the pattern of sex/age differences in scent application might reflect variation across species in the importance of predation to males, females and juveniles, or could be the result of differences in the function of applying substances.

Our data provided no support for the ectoparasite defence hypothesis. Flea loads were higher in juveniles than adults but juveniles did not SSA more than adult females, and individual flea load did not correlate positively with SSA duration. California ground squirrels had higher flea loads than rock squirrels, but we found no species difference in SSA behaviour.

Support was similarly weak for the conspecific deterrence hypothesis. Even though California ground squirrel males engaged in more aggressive interactions than rock squirrel males, they did not differ in SSA quantity. Similarly, we found no significant sex differences in aggression among adults of either species, but adult males applied snake scent less than did adult females in both species. Nevertheless, we only tested one specific prediction of the potential social function of SSA. It is possible that adult females and juveniles use snake scent in other social contexts. For example, they may apply predator scent to alert conspecifics to the presence of a predator or, in the case of California ground squirrels, may use snake scent to repel infanticidal conspecific females (Trulio et al. 1986; Trulio 1996) from their burrows.

Overall, our results indicate that SSA most likely serves an antipredator function for ground squirrels. Experiments are currently under way to assess the impact of snake scent on predators, ectoparasites and conspecifics. If these experiments similarly support the antipredator hypothesis, SSA would prove to be a novel form of defence behaviour in vertebrates. Chemical defences are ubiquitous among invertebrates, and several invertebrate and vertebrate species sequester the chemicals used in defence (e.g. Daly 1997; Williams et al. 2004). However, no vertebrate has clearly been demonstrated to use a self-applied chemical from a foreign source in predator defence.

## Supplementary Material

Supplementary material for this article can be found in the online version at doi: 10.1016/j.anbehav.2007.05.024.

## References

Bagg, A. M. 1952. Anting not exclusively an avian trait. *Journal of Mammalogy*, **33**, 243.

Baier, B., Lamml, M. & Kramer, B. 2006. Ontogeny of the electric organ discharge in two parapatric species of the dwarf stone-basher, *Pollimyrus castelnaui* and *P. marianne* (Mormyridae, Teleostei). *Acta Zoologica*, **87**, 209–214.

**Baker, M.** 1996. Fur rubbing: use of medicinal plants by capuchin monkeys (*Cebus capucinus*). *American Journal of Primatology*, **38**, 263–270.

**Bartram, S. & Boland, W.** 2001. Chemistry and ecology of toxic birds. *Chembiochem*, **2**, 809–881.

**Bernier, U. R., Furman, K. D., Kline, D. L., Allan, S. A. & Barnard, D. R.** 2005. Comparison of contact and spatial repellency of catnip oil and N, N-diethyl-3 methylbenzamide (Deet) against mosquitoes. *Journal of Medical Entomology*, **42**, 306–311.

**Berridge, K. C.** 1990. Comparative fine structure of action: rules of form and sequence in the grooming patterns of six rodent species. *Behaviour*, **113**, 1–22.

**Biardi, J. E.** 2000. Adaptive variation and coevolution in California ground squirrel (*Spermophilus beecheyi*) and rock squirrel (*Spermophilus variegatus*) resistance to rattlesnake venom. Ph.D. thesis, University of California, Davis.

**Birkinshaw, C. R.** 1999. Use of millipedes by black lemurs to anoint their bodies. *Folia Primatologica*, **70**, 170–171.

**Blumstein, D. T., Evans, C. & Daniels, J. C.** 2006. JWatcher 1.0. Available at http://www.jwatcher.ucla.edu/

**Boellstorff, D. E. & Owings, D. H.** 1995. Home range, population structure, and spatial organization of California ground squirrels. *Journal of Mammalogy*, **76**, 551–561.

**Brodie, E. D.** 1977. Hedgehogs use toad venom in their own defense. *Nature*, **268**, 627–628.

**Bursten, S. N., Kimsey, R. B. & Owings, D. H.** 1997. Ranging of male *Oropsylla montana* fleas via male California ground squirrel (*Spermophilius beecheyi*) juveniles. *Journal of Parasitology*, **83**, 804–809.

**Bursten, S. N., Berridge, K. C. & Owings, D. H.** 2000. Do California ground squirrels (*Spermophilus beecheyi*) use ritualized syntactic cephalocaudal grooming as an agonistic signal? *Journal of Comparative Psychology*, **114**, 281–290.

**Campbell, C. J.** 2000. Fur rubbing behavior in free-ranging black-handed spider monkeys (*Ateles geoffroyi*) in Panama. *American Journal of Primatology*, **51**, 205–208.

**Carroll, J. F., Kramer, M., Weldon, P. J. & Robbins, R. G.** 2005. Anointing chemicals and ectoparasites: effects of benzoquinones from millipedes on the lone star tick, *Amblyomma americanum*. *Journal of Chemical Ecology*, **31**, 63–75.

**Clark, L.** 1990. Starlings as herbalists: countering parasites and pathogens. *Parasitology Today*, **6**, 358–360.

**Clark, L. & Mason, J. R.** 1988. Effect of biologically-active plants used as nest material and the derived benefit to starling nestlings. *Oecologia*, **77**, 174–180.

**Coss, R. G.** 1999. Effects of relaxed natural selection on the evolution of behavior. In: *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms* (Ed. by S. A. Foster & J. A. Endler), pp. 180–208. Oxford: Oxford University Press.

**Craig, A.** 1999. Anting in afrotropical birds: a review. *Ostrich*, **70**, 203–207.

**D'Have, H., Scheirs, J., Verhagen, R. & De Coen, W.** 2005. Gender, age and seasonal dependent self-anointing in the European hedgehog *Erinaceus europaeus*. *Acta Theriologica*, **50**, 167–173.

**Daly, J. W.** 1997. Thirty years of discovering arthropod alkaloids in amphibian skin. *Journal of Natural Products*, **61**, 162–172.

**Drea, C. M., Vignieri, S. N., Cunningham, S. B. & Glickman, S. E.** 2002. Responses to olfactory stimuli in spotted hyenas (*Crocuta crocuta*): I. Investigation of environmental odors and the function of rolling. *Journal of Comparative Psychology*, **116**, 331–341.

**Dumbacher, J. P. & Fleischer, R. C.** 2001. Phylogenetic evidence for colour pattern convergence in toxic pitohuis: Mullerian mimicry in birds? *Proceedings of the Royal Society of London, Series B*, **268**, 1971–1976.

**Dunford, C.** 1977. Social system of round-tailed ground squirrels. *Animal Behaviour*, **25**, 885–906.

**Fauth, P. T., Krementz, D. G. & Hines, J. E.** 1991. Ectoparasitism and the role of green nesting material in the European starling. *Oecologia*, **88**, 22–29.

**Fitch, H. S.** 1948. Ecology of the California ground squirrel on grazing lands. *American Midland Naturalist*, **39**, 513–596.

**Fitch, H. S. & Twining, H.** 1946. Feeding habits of rattlesnakes. *Copeia*, **2**, 64–71.

**Gompper, M. E. & Hoylman, A. M.** 1993. Grooming with trattinnickia resin: possible pharmaceutical plant use by coatis in Panama. *Journal of Tropical Ecology*, **9**, 533–540.

**Gwinner, H., Oltrogge, M., Trost, L. & Nienaber, U.** 2000. Green plants in starling nests: effects on nestlings. *Animal Behaviour*, **59**, 301–309.

**Harrup, B.** 1992. Robin 'anting' with millipede. *British Birds*, **85**, 189.

**Hauser, D. C.** 1964. Anting by grey squirrels. *Journal of Mammalogy*, **45**, 136–138.

**Hemmes, R. B., Alvarado, A. & Hart, B. L.** 2002. Use of California bay foliage by wood rats for possible fumigation of nest-borne ectoparasites. *Behavioral Ecology*, **13**, 381–385.

**Hennessy, D. F. & Owings, D. H.** 1988. Rattlesnakes create a context for localizing their search for potential prey. *Ethology*, **77**, 317–329.

**Herron, R. M., Castoe, T. A. & Parkinson, C. L.** 2004. Sciurid phylogeny and the paraphyly of Holarctic ground squirrels (*Spermophilus*). *Molecular Phylogenetics and Evolution*, **31**, 1015–1030.

**Husak, M. S. & Husak, J. F.** 1997. Anting by a scissor-tailed flycatcher. *Southwestern Naturalist*, **42**, 351–352.

**Johnson, K.** 1981. Social-organization in a colony of rock squirrels (*Spermophilus variegatus*, Sciuridae). *Southwestern Naturalist*, **26**, 237–242.

**Kobayashi, T.** 2000. Behavioral responses of Siberian chipmunks toward conspecifics' applied snake scent. *Zoological Science*, **17**, 319–321.

**Kobayashi, T. & Watanabe, M.** 1986. An analysis of snake-scent application behavior in Siberian chipmunks (*Eutamias sibiricus asiaticus*). *Ethology*, **72**, 40–52.

**Lestel, D. & Grundmann, E.** 1999. Tools, techniques and animals: the role of mediations of actions in the dynamics of social behaviours. *Information sur Les Sciences Sociales*, **38**, 367–407.

**Mateo, J. M.** 2007. Ontogeny of adaptive behaviors. In: *Rodent Societie* (Ed. by J. O. Wolff & P. W. Sherman), pp. 195–206. Chicago: University of Chicago Press.

**Milton, S. J. & Dean, W. R. J.** 1999. The selective use of green aromatic plants in karoo bird nests. *Ostrich*, **70**, 243–245.

**Osborn, S. A. H.** 1998. Anting by an American dipper (*Cinclus mexicanus*). *Wilson Bulletin*, **110**, 423–425.

**Owings, D. H. & Coss, R. G.** 1977. Snake mobbing by California ground squirrels: adaptive variation and ontogeny. *Behaviour*, **62**, 50–69.

**Owings, D. H. & Leger, D. W.** 1980. Chatter vocalizations of California ground-squirrels: predator-role and social-role specificity. *Zeitschrift für Tierpsychologie*, **54**, 163–184.

**Owings, D. H., Virginia, R. & Paussa, D.** 1979. Time budgets of California ground squirrels during reproduction. *Southwestern Naturalist*, **24**, 191–195.

**Owings, D. H., Coss, R. G., McKernon, D., Rowe, M. P. & Arrowood, P. C.** 2001. Snake-directed antipredator behavior of rock squirrels (*Spermophilus variegatus*): population differences and snake-species discrimination. *Behaviour*, **138**, 575–595.

**Parkes, K. C., Weldon, P. J. & Hoffman, R. L.** 2003. Polydesmidan millipede used in self-anointing by a strong-billed woodcreeper

(*Xiphocolaptes promeropirhyncus*) from Belize. *Ornitologia Neotropica*, **14**, 1–2.

**Poran, N. S., Coss, R. G. & Benjamini, E.** 1987. Resistance of California ground squirrels (*Spermophilus beecheyi*) to the venom of the northern Pacific rattlesnake (*Crotalus viridis oreganus*): a study of adaptive variation. *Toxicon*, **25**, 767–777.

**Rodgers, J. A., Wenner, A. S. & Schwikert, S. T.** 1988. The use and function of green nest material by wood storks. *Wilson Bulletin*, **100**, 411–423.

**Ropartz, P.** 1968. The relation between olfactory stimulation and aggressive behaviour in mice. *Animal Behaviour*, **16**, 97–100.

**Rowe, M. P. & Owings, D. H.** 1978. Meaning of sound of rattling by rattlesnakes to California ground squirrels. *Behaviour*, **66**, 252–267.

**Salmon, T. P. & Marsh, R. E.** 1989. California ground squirrel trapping influenced by anal-gland odors. *Journal of Mammalogy*, **70**, 428–431.

**Schwagmeyer, P. L.** 1981. Alarm calling behavior of the 13-lined ground squirrel, *Spermophilus tridecemlineatus*. *Behavioral Ecology and Sociobiology*, **7**, 195–200.

**Sherman, P. W.** 1977. Nepotism and the evolution of alarm calls. *Science*, **197**, 1246–1253.

**Shriner, W. M. & Stacey, P. B.** 1991. Spatial relationships and dispersal patterns in the rock squirrel, *Spermophilus variegatus*. *Journal of Mammalogy*, **72**, 601–606.

**Stachowicz, J. J. & Hay, M. E.** 1999. Reducing predation through chemically mediated camouflage: indirect effects of plant defences on herbivores. *Ecology*, **80**, 495–509.

**Swaisgood, R. R., Rowe, M. P. & Owings, D. H.** 2003. 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. *Behavioral Ecology and Sociobiology*, **55**, 22–31.

**Tinbergen, N.** 1952. Derived activities: their causation, biological significance, origin and emancipation during evolution. *Quarterly Review of Biology*, **27**, 1–32.

**Trulio, L. A.** 1996. The functional significance of infanticide in a population of California ground squirrels (*Spermophilus beecheyi*). *Behavioral Ecology and Sociobiology*, **38**, 97–103.

**Trulio, L. A., Loughry, W. J., Hennessy, D. F. & Owings, D. H.** 1986. Infanticide in California ground squirrels. *Animal Behaviour*, **34**, 291–294.

**Tucker, A. O. & Tucker, S. S.** 1988. Catnip and the catnip response. *Economic Botany*, **42**, 214–231.

**Valderrama, X., Robinson, J. G., Attygalle, A. B. & Eisner, T.** 2000. Seasonal anointment with millipedes in a wild primate: a chemical defense against insects. *Journal of Chemical Ecology*, **26**, 2781–2790.

**Vandermeer, R. K. & Wojcik, D. P.** 1982. Chemical mimicry in the myrmecophilous beetle, *Myrmecaphodius excavaticollis*. *Science*, **218**, 806–808.

**Weldon, P. J.** 2004. Defensive anointing: extended chemical phenotype and unorthodox ecology. *Chemoecology*, **14**, 1–4.

**Weldon, P. J., Aldrich, J. R., Klun, J. A., Oliver, J. E. & Debboun, M.** 2003. Benzoquinones from millipedes deter mosquitoes and elicit self-anointing in capuchin monkeys (*Cebus* spp.). *Naturwissenschaften*, **90**, 301–304.

**Williams, B. L., Brodie, E. D., Jr & Brodie, E. D., III** 2004. A resistant predator and its toxic prey: persistence of newt toxin leads to poisonous (not venomous) snakes. *Journal of Chemical Ecology*, **30**, 1901–1919.

**Xu, Z. J., Stoddart, D. M., Ding, H. B. & Zhang, J.** 1995. Self-anointing behavior in the rice-field rat, *Rattus rattoides*. *Journal of Mammalogy*, **76**, 1238–1241.

**Zimen, E.** 1981. *The Wolf: His Place in the Natural World*. London: London Souvenir.

**Zito, M., Evans, S. & Weldon, P. J.** 2003. Owl monkeys (*Aotus* spp.) self-anoint with plants and millipedes. *Folia Primatologica*, **74**, 159–161.