Behavioral Ecology
doi:10.1093/beheco/arn046
Advance Access publication 25 April 2008

Programmed versus stimulus-driven antiparasitic grooming in a desert rodent

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We tested 2 hypotheses concerning regulation of grooming in flea-infested rodents and examined if 2 grooming components, scan and scratch grooming, are controlled by programmed and stimulus-driven regulation, respectively. The programmed grooming hypothesis proposes central programming that periodically evokes a bout of grooming to remove ectoparasites before they are attached and predicts that juvenile rodents 1) regardless of infestation status will invest more time in grooming (the body size principle) and 2) sustain lower flea densities than adults. The stimulus-driven grooming hypothesis postulates a direct response to irritation from ectoparasite bites and predicts that under flea-free conditions, 1) the stimulus-driven grooming regulation will not be activated, thereby neither juveniles nor adults will engage in grooming, but under flea infestation, 2) adults will invest more time in grooming than juveniles and sustain similar flea densities. We recorded the behavior of adult and juvenile flea-parasitized and nonparasitized rodents and quantified the frequency and duration of the 2 grooming components. Flea infestation increased the time devoted to grooming, supporting the existence of a regulation mechanism. However, the results did not support the dominance of neither hypothesis. Both forms of grooming were affected similarly by flea infestation and host age, hence may not necessarily be linked to a given regulation mechanism. Regardless of infestation status, time devoted to grooming was lower in juveniles, and both age groups sustained fleas at similar densities. We suggest that the assumptions and predictions of the 2 hypotheses should take into account the morphology and natural history of the host organism. *Key words:* body size principle, ectoparasites, programmed grooming, stimulus-driven grooming, time budget. *[Behav Ecol 19:929–935 (2008)]*

Grooming plays various roles in the health care, reproduction, and social life of an individual vertebrate (e.g., Wilkinson 1986; Sachs 1988; McLean and Speakman 1997). In particular, ectoparasite removal is considered to be the major purpose of grooming in wild animals (reviewed in Hart 1990; Mooring et al. 2002). Antiparasitic grooming is one of the most frequently performed defense behaviors in mammals and birds and is considered to be a major host-originated factor of mortality of hematophagous ectoparasites (see reviews by Marshall 1981; Clayton and Cotgreave 1994; Hart 1997a). Nevertheless, despite comprehensive evidence for the neurophysiological regulation of grooming, on the one hand, and detailed descriptions of the behavioral repertoires of grooming, on the other hand, the link between the neurophysiological mechanisms and the behavioral processes of antiparasitic grooming is not fully understood (Mooring et al. 2004).

There are 2 main nonmutually exclusive models for the neurophysiological regulation of antiparasitic grooming. The first model, programmed grooming, assumes a type of central programming (ultradian clock or endogenous generator) that periodically evokes a bout of grooming in order to remove ectoparasites before they are able to attach and blood feed (Hart et al. 1992; Mooring 1995). Although largely untested, it is likely that the frequency of the programmed grooming is modulated by chemical cues (acting on a "grooming center" in the central nervous system) that signal increased vulnerability to ectoparasite infestation (Hart 1997b). According to this model, a host is expected to invest in antiparasitic grooming on a regular basis even in an ectoparasite-free environment (Hart et al. 1992; Mooring and Samuel 1998a; Mooring, Hart, et al. 2006). Moreover, it is assumed that programmed grooming evolved to balance the costs of parasites against the costs of grooming. These costs may vary among host individuals depending on body size, reproductive status, and ecological conditions, and therefore, programmed grooming rate is expected to be affected by these variables (the body size, vigilance, and habitat principles; Hart et al. 1992). In particular, assuming equal efficiency of grooming between the different host groups, the programmed grooming model predicts that smaller bodied hosts (body size principle) and hosts exposed to high densities of ectoparasites (habitat principle) will groom at a higher rate than hosts of larger body size or inhabiting ectoparasite-poor habitat, while breeding males competing for access to females will groom less frequently compared with nonbreeding males (vigilance principle).

The second model, stimulus-driven grooming, postulates a peripheral mechanism that is a direct response to cutaneous irritation from ectoparasite bites (Wikel 1984; Alexander 1986; Wakelin 1996). Such peripheral control is influenced by the hypersensitivity response of the tissue immune system, whereby histamine is released from dermal mast cells at the site of the bite (Wikel 1984; Wakelin 1996). This hypersensitive irritation occurs after ectoparasites have attached and started to blood feed from the host. According to this model,

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Received 11 January 2008; revised 19 March 2008; accepted 22 March 2008.

in the absence of a stimulus, an animal is not expected to invest in antiparasitic grooming (Wakelin 1996). Similarly, the amount of neurostimuli is not related to body size, reproductive status, or ecological conditions, and thus, differences in stimulus-driven grooming rate between individuals should reflect only variations in infestation level (i.e., amount of stimuli). Both programmed and stimulus-driven mechanisms must operate concurrently. It is, however, of interest to determine whether in natural systems, grooming is regulated primarily by stimulus-independent central programming, by sensory input from ectoparasites, or both. It is also important to understand if different grooming components are controlled by separate regulation mechanisms.

A series of behavioral studies has provided broad support for the predictions of the programmed grooming model and suggested that programmed grooming predominates in the natural environment (see review in Mooring et al. 2004). However, these studies were based mainly on observations on ungulates (antelopes, cervids, domestic goats, and sheep; Hart et al. 1992; Mooring and Samuel 1998a; Mooring et al. 2000, 2002, 2004; Hart and Pryor 2004). Therefore, the generality of the patterns found in these studies needs to be confirmed by experimental studies on nonungulates. This will allow a general assessment of the role of programmed grooming as opposed to stimulus-driven grooming in various animals.

Here, we tested the 2 nonmutually exclusive hypotheses concerning regulation of antiparasitic grooming in rodents (*Meriones crassus*) parasitized by fleas (*Xenopsylla conformis*). Rodents are a convenient model to assess the relative role of programmed versus stimulus-driven antiparasitic grooming because the neurobiological basis of the programmed mechanism has been explored extensively in these animals (reviewed in Fentress 1988; Sachs 1988), and it has been shown that the main function of grooming in rodents is ectoparasite removal (Bell et al. 1962; Murray 1987; Levin and Fish 1998; Hawlena et al. 2007). However, to the best of our knowledge, there has been no attempt to test the programmed and stimulus-driven hypotheses using behavioral studies of rodents.

We tested the 2 hypotheses regarding regulation mechanisms of antiparasitic grooming by monitoring the grooming behavior of juvenile and adult rodents before and after flea infestation. We predicted that if antiparasitic grooming in rodents is programmed, then 1) regardless of infestation status, juvenile rodents will groom more frequently and for longer durations because parasite infestation is more costly for juveniles than for adults due to greater surface-to-volume ratio, which causes them to lose greater blood volume per unit of body mass (i.e., body size principle; Mooring et al. 2000). The higher susceptibility of juveniles to flea infestation compared with adults was supported in our previous study (Hawlena et al. 2006). Age-dependent rates of grooming may take place, for example, if an increase in growth hormone acting on a grooming center in the central nervous system. Consequently, 2) juvenile rodents will sustain lower flea numbers per unit body surface than adults. We also explicitly tested the assumption of the "body size principle" that juvenile and adult grooming are equally efficient in removing fleas. Alternatively, if grooming in rodents is mostly stimulus driven, then under flea-free conditions, 1) the stimulus-driven grooming regulation will not be activated, thereby neither juveniles nor adults will engage in grooming but 2) flea infestation will generate age differences in grooming as adult rodents which are infested by more fleas than juveniles (equal flea numbers per surface area but larger surface area compared with juveniles) will be prone to more flea stimuli and thus will groom more frequently and for longer duration than juveniles and sustain similar flea numbers per unit body surface.

Rodents use 2 major components of grooming, scan and scratch grooming, differ in the rate, form, and order of actions, involving different organs, and, most likely, belong to different branch of hierarchical organization (Sachs 1988 and references therein). Scan grooming is a routine ritual that has a clear cephalocaudal sequence (equivalent to "mouthing" or "forepaw grooming" in Sachs 1988). Scratch grooming is characterized by very fast unpredictable movements of the extremities toward a single region of the body and do not have a characteristic sequence (equivalent to "hindpaw scratching" in Sachs 1988). The documented differences between the 2 components of grooming raise a second question, namely, are these components regulate by the same mechanism? We examined this question by quantifying the frequency and duration of each grooming component separately. As scan grooming is a quite predictable and an organized behavior that target different regions of the body, we hypothesized that it is mainly controlled by a programmed mechanism. Conversely, scratch grooming is neither organized nor predictable but usually directed toward specific regions of the body, thereby, we hypothesized that it is mainly controlled by stimulus-driven mechanism. Observations on ungulates provide some evidence that scratch grooming (i.e., grooming with the hind leg limb) is indeed less influenced by central control than oral grooming, which may correspond to scan grooming in rodents (Mooring 1995; Mooring and Hart 1997; Mooring et al. 2004). Consequently, we predicted that the pattern of scan grooming will be in line with predictions of the programmed model of grooming regulation, whereas scratch grooming will be in line with predictions of the stimulus-driven model of grooming regulation.

MATERIALS AND METHODS

Animals

Rodents

Meriones crassus Sundevall is a common nocturnal rodent species of southern Israel. We used rodents from our laboratory colony. Progenitors of the colony were captured at the Ramon erosion cirque, Negev Highlands, Israel (30°58′N, 34°79′E), in 1996. We used only immune-naive male rodents to control for the possible parasite experience (Alexander 1986) and sexual (Moore 1986; Hart 1997b) biases. Adult hosts were at least 6 months old and were maintained individually in $20 \times 30 \times 10$ cm^3 plastic cages both prior and during the experiment to ensure that they are not reproductively active (Mooring, Patton, et al. 2006). They were kept on sand bedding in an animal room with an air temperature of 25 °C and photoperiod of 12:12 h light:dark and were provided daily millet seeds ad lib and alfalfa as a water source. The animal room was flea free, controlling for the effect of immunity or past flea stimulus. Juvenile rodents were separated from their mothers 30 days post partum and after 3 days in a cage with the other siblings were placed individually in the experimental cages at the same conditions than adult rodents. Rodents at this age are weaned and have a full coat but still are not reproductively mature. Prior to the experiment, all rodents were allowed 3 days of acclimation in the experimental cages within the animal room, where the experiment took place. During the experiment, rodents were housed individually in glass cages ($40 \times 20 \times 20$ cm^3) to enable clear observation of their behavior. The floor in each cage was covered by 1 cm sand, and the cage contained a small plastic, transparent nest-box and food ad lib (millet seeds and alfalfa as a water source). The sidewalls of each cage were covered with opaque paper to prevent eye contact between adjacent animals. Cages were located in the darkened room with a dim red light shining

into the cages from the front. All observations were conducted by the same person sitting silently and motionless 2 m away from the experimental cages.

Fleas

Meriones crassus is naturally parasitized by several flea species, but *X. conformis* predominates in terms of intensity of infestation (Krasnov et al. 1996, 1997, 1998). Fleas were obtained from a laboratory colony initiated from field-collected specimens of *M. crassus*. Rodent hosts were placed in plastic cages containing a steel nest-box with a screen floor and a pan with a mixture of sand and dried bovine blood (larvae nutrient medium) on the bottom. Gravid female fleas left the host and deposited eggs in this substrate, where development of larvae took place. Every 2 weeks, all substrate and bedding material were collected from the nest-box and transferred into an incubator, where flea development and emergence took place at 25 °C and 75% relative humidity (RH). The newly emerged fleas were placed on clean animals. Colonies of fleas were maintained at 25 °C and 75% RH with a photoperiod of 12:12 h dark:light.

Experimental design

Thirty-seven adult and 37 juvenile males of *M. crassus* were subjected randomly to 2 treatments. Of these, 24 juveniles and 24 adults were infested by fleas (hereafter, the parasitized group) while the rest served as a control group. Each experimental trial lasted for 2 consecutive nights with a similar experimental procedure. However, during the first night of the observations, no fleas were introduced, whereas on the second night, rodents from the parasitized group were infested by fleas. This experimental design allowed us to control for temporal (comparison of the control group in the first and second days) and individual (comparison of the same individual with and without fleas) variation in behavior while isolating the antiparasitic element of grooming. We recorded the behavior of rodents at night (see below). To quantify the effect of the length of infestation period on flea density (i.e., a measure of grooming efficiency), we varied the time of flea infestation by randomly choosing one juvenile and one adult rodents from the parasitized group at the end of each hourly observation and counting the surviving fleas (see below). The behavioral observation on the chosen individuals was then terminated to avoid changes in the rodent's behavior due to our intervention. This design allowed us to quantify grooming efficiency in the parasitized group while avoiding any interference during the behavioral observations.

Flea manipulation

Fleas were applied to the treated rodents at the onset of the second night of each trial, 15 min prior to the first observation. Numbers of fleas were within the natural infestation range of wild Sundevall's jirds (Krasnov et al. 2003; Shenbrot G, unpublished data) and resulted in a density of 0.4 fleas/cm^2 body surface area for both juveniles and adults (where surface area was calculated according to Meeh equation for mammals; Stahl 1967). At the end of each hourly observation, we brushed the fleas from the body of the chosen juvenile and adult rodents over a white plastic can, checked their cage for jumped-off fleas, and then counted the surviving fleas. In this method, at least 95% of the fleas are recovered (Hawlena H, unpublished data). At the end of the experiment, rodents were placed back into their original cages and provided with seeds and alfalfa ad libitum. The experimental protocol met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel and was approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments.

Behavioral observations

The observer recorded the rodents' behavior every minute during the first 30 min of each hour between 2000 and 0800 h. We distinguished between the 2 principal forms of grooming behavior: scan grooming and scratch grooming. Scan grooming is a routine ritual characterized by paw movement and mouthing and have a clear cephalocaudal sequence, starting with nose wipes by the forepaw and ending with anogenital and tail grooming. Scratch grooming is characterized by very fast unpredictable movements of the extremities, usually the hindpaw, toward a single region of the body and do not have a characteristic sequence. In some instances, rodents were using fast, unpredictable oral grooming of specific regions that could not be reached by the paws and were recorded as scratch grooming as well.

As rodents scan groom and scratch groom very fast and the change between different episodes of a single grooming movement may occur within seconds, we could not reliably count the actual grooming movements. Instead, we focused on each connected series of episodes, hereafter, grooming bouts. A grooming bout was considered to be started when either scan or scratch grooming was observed and were considered to be terminated when a nongrooming activity was started, a transition to the second form of grooming took place, or at the end of the observation period. Truncation of the bouts at the end of the observation period may cause an underestimation of grooming time, but this was assumed to affect estimations of both scratch and scan grooming in a similar way. Bout frequency (number of bouts per observation) and duration (average time of bouts per observation) of each form of grooming were later calculated, and the reported data was recalculated as frequency or duration of grooming per hour.

Data analysis

We applied repeated measures analysis of covariance (ANCOVA) to test for the effect of host age on the change in flea densities between the beginning and the end of a trial. As grooming is expected to reduce flea densities on the host, low flea densities at the end of the trial corresponded to high grooming efficiency. The length of the infestation period (time between flea addition and removal) was used as a covariate and flea density at the beginning and at the end of a trial was the repeated variable.

We included in the behavioral analysis 54 individuals (adults: 13 control and 14 parasitized; juveniles: 13 control and 14 parasitized) that were observed during 6 h (between 2000 and 0200 h). Observations for the additional 20 individuals were interrupted before 0200 h for flea removal and therefore could not be included in the analysis. We applied repeated measures analysis of variance to test for the effect of age and infestation (between-groups factors) on frequency and duration of the 2 forms of grooming, where activity (scan vs. scratch grooming) and time (first vs. second night) served as repeated factors. We applied homogeneity of slope models to test whether frequency and duration of scan and scratch grooming (covariates) are good predictors for flea density at the end of a trial (dependent variable) and whether the 2 grooming components are equally efficient in flea removal by juveniles and adults (e.g., age was independent variable).

The frequency and duration of scan and scratch grooming were log transformed prior to analysis to adjust deviations from normality. All statistical tests were 2 tailed. Data are presented as means ± standard error before log transformation.

RESULTS

Flea addition significantly increased frequency but not duration of the 2 forms of grooming (as indicated by the time × infestation interactions in Table 1, Figure 1). Prior to flea addition (i.e., first night), juvenile and adult rodents from the parasitized group invested a total time of 2.3 ± 0.34 and 3.4 ± 0.54 min/h, respectively, in scan grooming and 1.2 ± 0.24 and 2.1 ± 0.29 min/h, respectively, in scratch grooming. After flea addition, total time devoted to scan grooming was almost doubled (4.4 ± 0.53 and 7.1 ± 0.67 min/h in juvenile and adult rodents, respectively) and time devoted to scratch grooming was more than tripled (4.6 ± 0.56 and 6.4 ± 0.71 min/h in juvenile and adult rodents, respectively).

The effect of the 4-way interaction (time × activity × infestation × age) on frequency and duration of grooming was not significant, indicating that the 2 forms of grooming are affected similarly by flea infestation and host age and therefore cannot be used to distinguish between the 2 models of grooming regulation (Table 1, Figure 1).

Host age had a significant effect on both frequency and duration of scan and scratch grooming (Table 1, Figure 1). Flea infestation had similar effects on frequency and duration of scan and scratch grooming in juveniles and adult rodents (as indicated by nonsignificant time × infestation × age interaction in Table 1, Figure 1). Both before and after flea infestation, adult rodents groomed at higher frequency and duration than juvenile hosts (Figure 1).

Frequency and duration of both scan and scratch grooming were good predictors for flea density at the end of the trial (homogeneity of slopes model; $60 > F > 9$, $P < 0.005$, for all tests; Figure 2). The relationships between duration of scan grooming and flea density at the end of a trial were stronger in juveniles than in adults (homogeneity of slopes model; $F = 6.2$, $P < 0.05$; Figure 2, lower right). However, the relationships between the other measures of grooming (i.e., frequency of scan grooming and frequency and duration of scratch grooming) and the flea density at the end of a trial were not significantly different between juveniles and adults (homogeneity of slopes model; $2.4 > F > 0.1$, $0.77 > P > 0.14$, for all tests; Figure 2).

The higher time investment of adults in grooming was not reflected in the antiparasitic efficiency of grooming. After removal of the effect of the infestation period, the changes in flea densities on adult and juvenile rodents between the beginning and the end of a trial were similar (repeated measures ANCOVA: $F = 0.77$, $P = 0.39$). By the end of a trial, juvenile rodents sustained 0.26 ± 0.021 fleas/cm^2, whereas adults sustained 0.29 ± 0.012 fleas/cm^2. However, the length of the infestation period had a strong negative effect on the flea density (repeated measures ANCOVA: $F = 18$, $P < 0.001$), indicating that grooming is efficient for flea removal. After 0.5 h, rodents removed 3.12% of the fleas, whereas after 11.5 h, 39.6% of the initial flea density were removed.

Table 1

Summary of repeated measures analyses of variance testing the effect of flea infestation and host age group (between-groups factors) on the frequency (number of bouts per hour) and duration (average time of bout per hour) of scan and scratch grooming (activity, repeated factor) during the first and second experimental nights (time, repeated factor)

	Grooming frequency		Grooming duration	
Effect	df	*F*	df	*F*
Infestation	1	6.6*	1	0.024
Age	1	18.7****	1	31.7****
Infestation × age	1	0.58	1	0.50
Error	50		50	
Time	1	32****	1	19.4****
Time × infestation	1	66****	1	3.6
Time × age	1	0.74	1	0.21
Time × infestation × age	1	0.85	1	0.82
Error	50		50	
Activity	1	8.0**	1	119****
Activity × infestation	1	5.8*	1	15.6****
Activity × age	1	0.016	1	3.1
Activity × infestation × age	1	1.8	1	0.85
Error	50		50	
Time × activity	1	2.4	1	0.14
Time × activity × infestation	1	5.0*	1	8.08**
Time × activity × age	1	11***	1	7.8**
Time × activity × infestation × age	1	0.61	1	3.0
Error	50		50	

Note that the parasitized groups of rodents were infested only on the second experimental night. df, degrees of freedom.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.005$.

****$P < 0.001$.

DISCUSSION

This study illustrates the importance of grooming in shaping the relationships between a host and its ectoparasites and supports the existence of some type of a regulation mechanism of grooming in rodents similar to birds (Moller 1991), bats (Giorgi et al. 2001), cats (Eckstein and Hart 2000b), and impalas (Mooring et al. 1996). We demonstrated experimentally that flea exposure increases grooming activity. Furthermore, a comparison of patterns of grooming in flea-free, as opposed to flea-parasitized rodents, allowed us to isolate the antiparasitic element of grooming. We showed that antiparasitic grooming of rodents is highly efficient in reducing flea densities on a given host and that both frequency and duration of grooming in rodents are good predictors for flea removal. We attempted to examine the relative role that programmed and stimulus-driven models play in regulation of grooming in 3 ways: 1) quantifying the time devoted to grooming under flea-free conditions, 2) looking separately on scan versus scratch grooming, and by 3) testing the body size principle. Our results did not support the dominance of one regulation mechanism over the other.

Rodents invested time in grooming even before flea infestation

According to the programmed grooming hypothesis, a host is expected to invest in antiparasitic grooming on a regular basis even in an ectoparasite-free environment (Hart et al. 1992; Mooring and Samuel 1998a). In contrast, the stimulus-driven hypothesis predicts that in the absence of a stimulus, an animal is not expected to invest in antiparasitic grooming (Wakelin 1996). Flea-free rodents invested on average 5.41 ± 0.38 min/h ($N = 54$) in scan and scratch grooming. This accounts for about 15% of their nonsleeping time and 9% of their total nocturnal time budget (Hawlena et al. 2007). Similar proportions of the time budget allocated for grooming have been observed in parasite-free bats (5.2%; Giorgi et al. 2001) and cats (8–9%; Eckstein and Hart 2000a, 2000b). However, recall that scan and scratch grooming in a parasite-free environment may be used for purposes other than ectoparasite removal (removal of dirt, debris, and excess oil and maintenance of the fur or feather and skin; reviewed in

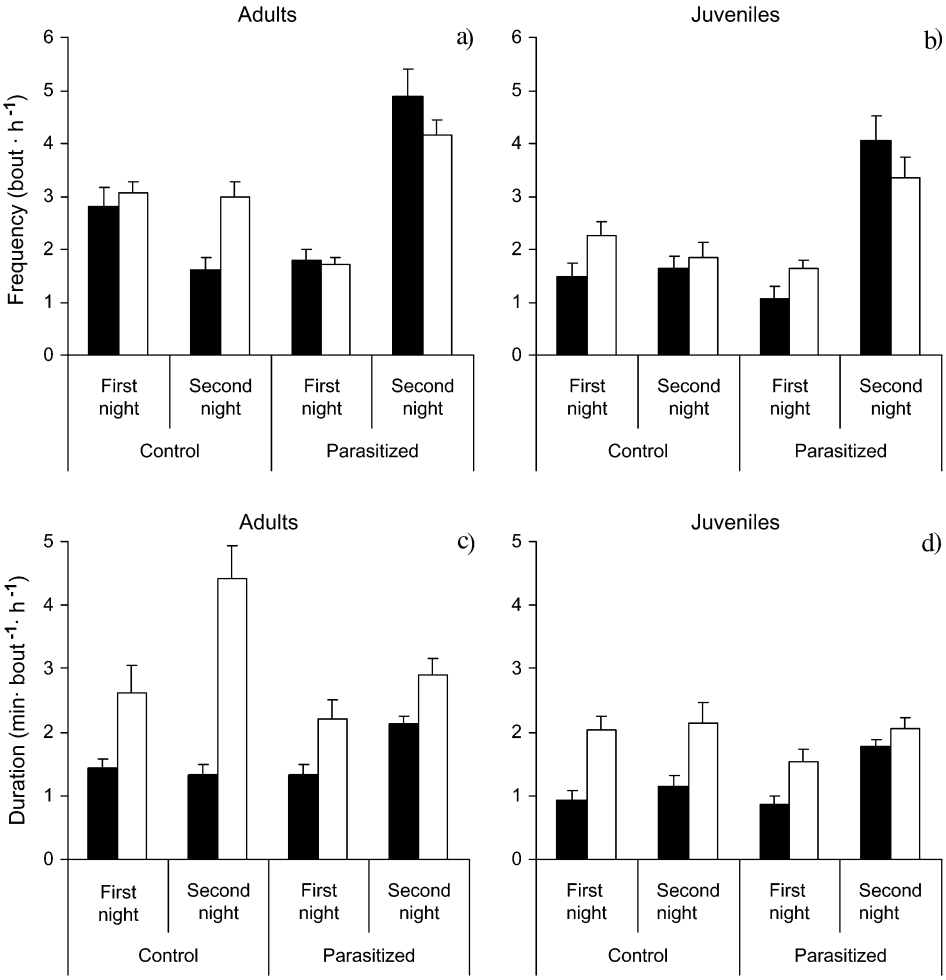

Figure 1
Mean (+standard error) frequency (bouts per hour) and duration (minutes per bouts per hour) of scratch grooming (black columns) and scan grooming (white columns) by control and flea-infested adult (a, c) and juvenile (b, d) rodents during the first and second experimental nights. Note that the parasitized groups of rodents were infested only on the second experimental night.

Sachs 1988), and thus, these observations do not necessarily support the programmed grooming hypothesis. For example, the time devoted to grooming changed over the 2 experimental days even in the control group (Figure 1a,c), most likely because of small changes in the cage environment that increased the need for fur cleaning (e.g., increase in the amount of food remains or feces). Note, that our experimental design, in which 1) individuals were randomly assigned to the 2 treatment groups and 2) each individual in the parasitized group was compared before and after flea infestation, rules out the possibility that differences between treatment groups are a result of differences in this nonparasite-removal grooming.

The 2 forms of grooming were affected similarly by flea infestation and host age

We attempted to examine the relative role that programmed and stimulus-driven models play in regulation of grooming by looking separately on scan versus scratch grooming that were predicted to be controlled by the programmed and stimulus-driven models, respectively. However, as the 2 forms of grooming were affected similarly by flea infestation and host age, it is likely that programmed and stimulus-driven grooming are not necessarily linked to a given grooming mode. The fact that flea-free rodents spent some time in scratch grooming further indicates that scratch grooming is not linked only to stimulus-driven grooming. Therefore, the separation of the 2 grooming components in rodents could not be used to distinguish between the 2 models of grooming regulation.

The body size principle was refuted

The most distinctive test of the programmed model is the comparison of grooming between juveniles and adults. We predicted that if the programmed grooming model operates in rodents, then the body size principle would be confirmed. In contrast to the body size principle, frequency and duration of scan and scratch grooming were lower in juvenile than in adult rodents and by the end of the trials the 2 age groups sustained fleas at similar densities. The latter results are in line with the third prediction of the stimulus-driven model, which predicts that under flea infestation, adults will invest more time in grooming than juveniles and sustain similar flea numbers per unit body surface. However, although the model predicts age differences merely under flea infestation, adult rodents in this study invested more time in grooming than juveniles under flea-free conditions as well.

The body size principle has been strongly supported in large mammals such as sheep, goats, deer, and antelopes (Mooring

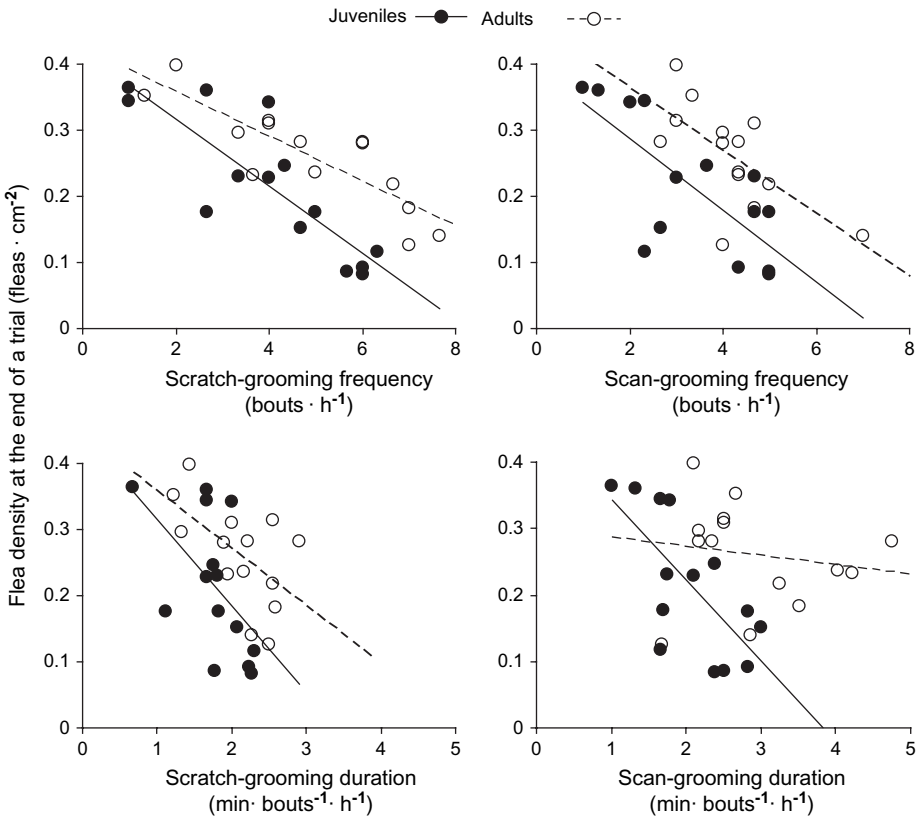

Figure 2
Relationships between frequency (bouts per hour, upper figures) and duration (minutes per bouts per hour, lower figures) of scan and scratch grooming and flea density at the end of a trial (fleas per square centimeter) in adults (empty circles, dashed line) and juvenile (full circles, solid line) parasitized rodents during the second experimental night.

and Hart 1997; Mooring and Samuel 1998b; Mooring et al. 2002; Hart and Pryor 2004). It therefore remains to be investigated why the body size principle does not hold for rodents. One possible distinction between rodents and the other studied organisms is the differences between the surface-to-mass ratio of juveniles and adults. In ungulates, when juveniles had only 150% greater surface-to-mass ratio than adults or less, their grooming rate no longer exceeded that of adults (Hart and Pryor 2004). Here, we used juvenile rodents as soon as they become independent of their parents since till then they are groomed by their mothers. At this time, their surface-to-mass ratio was 138% greater than this ratio for adults. In ungulates, the juveniles are fully capable of grooming almost as soon as they are born, and in nature, the newborn move about and are exposed to ectoparasites. Consequently, juvenile ungulates studied were still nursing from their mothers at the time their grooming rate was observed to exceed that of adults. It is therefore possible that the difference between the surface-to-mass ratio of juveniles and adult rodents has passed the threshold for effect on grooming. It is likely that the body size principle would not hold for other altricial mammals, such as cats, too, as their body size may progress fast enough that after they are fully capable of grooming the surface-to-mass ratio has passed the threshold for effect on grooming. Another possible distinction between rodent and ungulate models is the dominant group of ectoparasites that infests and impacts the inclusive fitness of the host. Ungulates are mostly parasitized by ticks (Mooring and Samuel 1998b), whereas the most abundant ectoparasites in rodents in terms of both intensity and prevalence are fleas. Periodic bouts of programmed grooming, delivered rather randomly across the body surface, are likely to maintain a low population of ticks, which are slow moving, whereas such grooming may be less effective against fleas, which are highly mobile, and thus can easily escape from the area being groomed. In contrary, stimulus-driven grooming, delivered quickly to the site as the flea is biting, may be more effective against fleas.

The "threshold" and the "ectoparasite group" explanations cannot fully explain the discrepancy between the rodent and ungulate systems because it does not clarify why juvenile rodents groomed less frequently and for shorter durations than adults, regardless of the infestation status. The lower grooming frequency and duration in juvenile compared with adult rodents are especially intriguing considering previous findings that juvenile rodents suffer higher mortality than adult rodents under similar flea densities (Hawlena et al. 2006). Three mutually nonexclusive explanations can be offered for the lower investment in grooming in juveniles compared with adult rodents. First, it is possible that the success of flea removal is positively related to the rodent's body surface. Consequently, adult hosts in our study that had on average 1.9 times the surface area of juvenile hosts are expected to groom 1.9 times longer than juvenile hosts to achieve the same density of fleas. Under the assumption of equal efficiency of juvenile and adult hosts in removing fleas, the 1.6-fold difference between total time (duration × frequency) devoted to grooming of adult and juvenile hosts seems to be close enough to the predicted 1.9. Second, our results suggest that in rodents, the assumption of the body size principle that juveniles and adults are equally efficient in flea removal is fulfilled only partly. Duration of scan grooming was not a good predictor of flea removal in adults (Figure 2, lower right) and

this might be the reason that at the end of the trial adults sustained similar flea densities to juveniles in spite of the longer time that they spent in grooming. Higher efficiency of juveniles could be, for example, a result of their shorter, less dense fur (Webb et al. 1990) that limits possible refuge for the fleas. Third, a trade-off between grooming and other activities such as sleeping and feeding may constrain the time devoted to grooming in juveniles but not in adult rodents (Hawlena et al. 2007), thereby juveniles may not be able to increase the time spent grooming to achieve even higher ectoparasite-removal efficiency than adults.

The refuting of the body size principle suggests that interspecific variation in host morphology (e.g., fur density, differences in the size of the grooming-operating organs, and manipulation abilities) and natural history (e.g., altricial vs. precocial organisms) may require a more species-specific consideration in tests of the assumptions as well as in construction of predictions of the programmed grooming model.

FUNDING

Ministry of Science, Culture and Sport of Israel (3-571).

Amos Bouskila was involved in interesting and stimulating discussions. We are grateful to Meital Yogev, Gil Ben Natan, and Arnon Tsairi for their invaluable help with all aspects of the study. Benjamin Hart and Michael Mooring provided many helpful comments on an earlier version of this paper. This is publication no. 607 of the Mitrani Department of Desert Ecology and no. 246 of the Ramon Science Center.

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