

## Sexually dimorphic grooming in bison: the influence of body size, activity budget and androgens

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(Received 9 November 2005; initial acceptance 26 January 2006;  
final acceptance 28 February 2006; published online 18 July 2006; MS. number: A10296)

The programmed grooming model proposes that animals groom in response to an internal timing mechanism that has evolved to control ectoparasites. This model predicts that polygynous males will groom less frequently than smaller females (sexually dimorphic grooming) because (1) parasitism is more costly for smaller individuals (body size principle), and (2) during the breeding season, sexual selection has favoured males that reduce grooming to enhance vigilance for mates and rivals (vigilance principle). The proximate mechanism for vigilance-mediated suppression of grooming may be either time budget conflicts (males are too busy breeding to groom) or physiological suppression (the action of elevated testosterone). In this study, we examined the influence of body size, breeding activity and androgens on sexually dimorphic grooming in American bison, *Bison bison*. Bison were observed before (pre-rut) and during the breeding season (rut) at National Bison Range (NBR) and Fort Niobrara (FTN) national wildlife refuges; androgens were measured in FTN males. Females oral-groomed two to five times more than males during pre-rut, and 5–40 times more than tending males (guarding oestrous females) during the rut; tending males oral-groomed less than pre-rut males at both sites. Rutting activity of tending males was much greater than that of nontending or nonrutting males, but all males groomed at the same low rate. Androgen levels increased between pre-rut and rut for all males, but androgen levels of tending males were higher than those of nontending males. In a subsample of males for whom both androgen and grooming data were available, androgen levels were negatively correlated with oral-grooming rate, as predicted by the physiological suppression model. We conclude that effects of body size and sexually selected vigilance work independently and additively to produce sexually dimorphic grooming in bison. The vigilance-associated suppression of grooming rate in breeding males appears to be due to the action of elevated testosterone rather than conflicting time budgets.

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Self-grooming (hereafter grooming) is commonly performed by mammals and other vertebrates to maintain the body surface and remove ectoparasites (Hart et al. 1992; Moore 2002). Ticks are the most important ectoparasites of wild animals, and are a major selection force for the evolution of grooming in the natural environment (Lightfoot & Norval 1981; Hart 1990; Allan 2001; Giorgi

et al. 2001). For example, growing cattle show tick-associated declines in growth (Little 1963; Sutherst et al. 1983; Norval et al. 1988; Kaiser et al. 1991), which could have fitness-compromising consequences for wild animals. Because ectoparasite infestation is costly, grooming behaviour that effectively removes ectoparasites should be selected for by natural selection. Experimental studies in which grooming was restricted have demonstrated the effectiveness of grooming in removing ectoparasites (Koch 1981, 1988; Hart 1990; Mooring et al. 1996a).

Two physiological mechanisms mediate tick-defence grooming: central control (Colbern & Gispén 1988; Fentress 1988; Spruijt et al. 1992) and peripheral stimulation (Riek 1962; Willadsen 1980; Wikel 1984). Previous studies indicate that centrally controlled grooming predominates

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over peripheral stimulation in wild ungulates (Hart et al. 1992; Mooring & Hart 1995b; Mooring et al. 1996a, b, 1998, 2000, 2002, 2004a; Mooring & Samuel 1998). The 'programmed grooming model' postulates a type of central programming that periodically evokes a bout of grooming so as to remove ticks preventively before they are able to attach and blood-feed (Hart et al. 1992). The rate at which programmed grooming is delivered is thought to be modulated by chemicals acting on receptors of a 'grooming control centre' in the central nervous system (CNS); such chemicals (e.g. histamine released during tick blood-feeding) would be associated with the cost of tick infestation (Hart 1997). Hormones and neuropeptides implicated in central control of grooming rate include testosterone, growth hormone, prolactin and vasopressin (Mori et al. 1985; Maeda et al. 1986; Meisenberg 1988; Mooring et al. 1998; Kakuma et al. 2003; Hart & Pryor 2004). Recently, Greer & Capocchi (2002) found that a mutant gene of *Hoxb8* triggered excessive self-grooming and allogrooming in mice, lending support to the concept of an intrinsic timing mechanism that regulates grooming rate.

The programmed grooming model predicts that females of polygynous species will groom more frequently than conspecific males (sexually dimorphic grooming) as the result of two mechanisms (Hart et al. 1992; Mooring et al. 2000, 2002). First, because surface area-to-mass ratio increases with declining body size, the 'body size principle' proposes that small animals incur higher costs for a given density of tick infestation compared to larger animals (Hart et al. 1992). Small animals provide relatively more surface area for tick attachment but relatively less blood volume for tick feeding. As a result, one tick sucks a greater proportion of blood from a smaller animal. Assuming equivalent rates of tick infestation, small-bodied animals should groom more frequently than large ones to maintain a lower density of ticks (Olubayo et al. 1993; Gallivan et al. 1995). The corollary is that similar-sized individuals should groom at a similar rate.

Second, because grooming distracts from vigilance (Maestripieri 1993; Cords 1995; Mooring & Hart 1995a), the 'vigilance principle' predicts that, during the breeding season, reproductively active males will groom less than females to maintain high levels of vigilance for rival males or oestrous females. Testosterone is believed to contribute to regulation of the programmed grooming rate, with higher levels of testosterone resulting in a physiological suppression of programmed grooming (Mooring et al. 1998, 2004a; Kakuma et al. 2003). Although studies have indicated support for the vigilance prediction (Hart et al. 1992; Mooring & Hart 1995b; Mooring et al. 1996b, 2002, 2004a), depressed grooming rate in sexually dimorphic males during the breeding season can also be explained by the body size principle. However, the vigilance principle predicts sexually dimorphic grooming rates independent of the body size effect.

The purpose of this study was to simultaneously test the predictions of the body size and vigilance principles in a large, sexually dimorphic herbivore, the American bison, *Bison bison*. Previous work has supported the body size prediction of the programmed grooming model for bison

outside of the breeding season (Mooring & Samuel 1998). Recent work has also demonstrated a dramatic elevation in male androgen levels during the rut compared with pre-rut, with tending bulls showing higher androgen levels than nontending ones (Mooring et al. 2004b). However, to tease apart the effects of body size and vigilance on sexually dimorphic grooming, it is necessary to simultaneously examine the influence of body size, activity budgets and androgen levels on grooming rates; this is the first study to do so.

Bison show male-dominance polygyny, and large size and fighting ability have been favoured in bison males by natural selection (Lott 2002). Sexual body size dimorphism is extreme: adult males ( $\bar{X}$  = 900 kg) are about twice the mass of females ( $\bar{X}$  = 450 kg); indeed, bison males are the largest land mammals native to the western hemisphere (Shaw & Meagher 2000). Outside of the breeding season (rut), adult male and female bison are segregated into separate social groups (Post et al. 2001; Mooring et al. 2005). During the rut, bison males aggregate with females and young in mixed herds and compete for the privilege of guarding (tending) and copulating with oestrous females. Most breeding takes place from mid-July to mid-August, with the peak around 1 August (Lott 1981; Meagher 1986). During the rut, males tend females by staying close to them until they are sexually receptive and then mating with them. A female may be tended by several males that alternately displace the previous male (Wolff 1998). During tending, males frequently perform a suite of consorting behaviours and displays, such as bellowing, scent-urination, pawing, rubbing and wallowing (although wallowing also has nondisplay functions outside of the rut; McMillan et al. 2000). If male 'vigilance' is associated with this suite of display and consorting behaviours, then, the body size and vigilance principles of the programmed grooming model make the following predictions.

(1) Outside of the rut, when sexual segregation occurs, males will groom less than females primarily because of the body size effect. In addition, outside of rut, the grooming rate of subadult males (similar in body size to females) will be more similar to that of females than to that of adult males.

(2) During the rut, when the sexes aggregate, actively breeding (tending) males will groom less than the females they are tending, and less than males outside of rut because of the additive effects of both the body size principle and the vigilance principle. Grooming rates of breeding males may be suppressed (1) because increased rutting conflicts with grooming time, and/or (2) because increased testosterone during the breeding season down-regulates the intrinsic rate of grooming by physiological means.

We observed the grooming rates of male and female bison outside of and during the breeding season to investigate whether the effects of body size and vigilance on sexually dimorphic grooming are independent and additive. To test these predictions, we observed the grooming rate of bison at two national wildlife refuges. Prior to the breeding season (pre-rut), we tested the body size prediction independent of the vigilance principle;

during the breeding season (rut), we examined the added effect of vigilance on grooming rate of breeding males. Activity budgets were recorded to describe breeding activity, and androgens were characterized by faecal steroid analysis.

## METHODS

### National Bison Range

Bison were studied at the National Bison Range (NBR), Moiese, Montana, U.S.A., during June–August 2002. The national wildlife refuge consists of 9000 ha (86 km<sup>2</sup>) of primarily palouse prairie, at elevations from 820 to 1500 m. Bison at NBR segregate into distinct social groups throughout most of the year. The oldest males ( $\geq 7$  years) are solitary, male groups are composed of males at least 2 years old, and mixed groups consist of females and their offspring as well as a few males (Berger & Cunningham 1994; Shaw & Meagher 2000; Post et al. 2001). Nursery herds of females and calves (without older males) form during the calving season. For this study, solitary and male groups are termed 'bull groups', while mixed and nursery groups are termed 'cow groups'. The bison population during summer 2002 numbered 420 adults, subadults and yearlings, plus young-of-year calves. All bison were branded on the right or left hindquarters with the last digit of the date of birth (the side alternating by decade), allowing us to know the age of all animals.

Because NBR bison were not individually marked, we sampled individuals from as many groups as possible to avoid repeat sampling. To avoid inflating the sample size, we limited the number of focal observations conducted to the number of individuals of each age/sex class in the herd. Every effort was made to avoid repeat sampling of the same individuals in a group based on brands and individual markings that could be recognized during an observation session. Bison were located by driving 4-wheel-drive vehicles along refuge roads and tracks during all daylight hours. Observations were made from the vehicle with 10 $\times$  binoculars and 15–60 $\times$  telescopes during 20-min focal animal samples (Altmann 1974). During 20-min samples, grooming behaviour was recorded continuously and activity budgets were measured by instantaneous sampling at 1-min intervals (Altmann 1974). Focal animal observations at NBR focused on 144 females (adult females  $\geq 3$  years old), 99 males (adult males  $\geq 4$  years old) and 53 subadult males (2–3 years old) in the herd.

For continuous recording of grooming behaviour, we distinguished between grooming 'episodes' and 'bouts'. An episode consisted of each individual grooming motion (i.e. tongue-lick or hindleg-scratch), whereas bouts were defined as an uninterrupted sequence of episodes separated from any subsequent bout by an interval of at least 5 s and/or a switch to another body region (bouts of a single episode were considered insect-repelling and discarded). Focal activity scans at 1-min intervals were used to compute the mean percentage of time that focal animals engaged in feeding, standing, moving, lying down,

ruminating and rutting behaviours. If the focal animal was engaged in more than one activity at a time (e.g. standing and ruminating), we recorded both activities. Wrist watches with repeating alarm function were used for timing instantaneous scans. Data were written directly into notebooks in the field and entered on laptop computers back at camp.

Interobserver reliability observations (Caro et al. 1979) for grooming were conducted by all observers (M.S.M., D.D.R., E.R.O., A.L.K.) watching the same animals at the same time. Correlation coefficients among observers were high for oral grooming (mean  $r = 0.88$ ; oral bouts mean  $r = 0.85$ , oral episodes mean  $r = 0.90$ ) and slightly less for scratch grooming (mean  $r = 0.75$ ; scratch bouts mean  $r = 0.72$ , scratch episodes mean  $r = 0.77$ ). Interobserver reliability for focal activity scans was high (overall mean  $r = 0.89$ ; feed:  $r = 0.99$ , stand:  $r = 0.97$ , lie:  $r = 0.98$ , walk:  $r = 0.89$ ).

### Fort Niobrara

We conducted fieldwork at Fort Niobrara National Wildlife Refuge (FTN) near Valentine, Nebraska, U.S.A., during June–August of 2004. The refuge consists of 7742 ha (77 km<sup>2</sup>) along the Niobrara River in the Sandhills of northcentral Nebraska. The bison herd is currently maintained at 350 head after the autumn roundup, and numbers approximately 475 following calving. All bison at FTN are individually marked with unique brands. Androgen levels were measured by faecal steroid analysis. Behavioural observations were conducted with 10 $\times$  binoculars from 4-wheel-drive vehicles, either from a trail or off-road. Observations at FTN were conducted using the same procedure as at NBR, except that all observations were conducted by one observer (M.S.M.). Analysis of FTN grooming and androgen data is based on means of individually known bison.

### Faecal Androgens

Faecal samples were collected from June to August 2004 from sexually mature bulls at FTN using the procedure of Mooring et al. (2004b). To summarize, samples were taken opportunistically during all daylight hours only when defecation was observed from a known individual. Fresh faecal material was transferred to a polypropylene container and placed immediately into an ice chest. Samples were transferred to a freezer at  $-20^{\circ}\text{C}$  for storage until they could be shipped overnight on dry ice to the laboratory at Conservation and Research for Endangered Species (CRES). At the laboratory, samples were lyophilized, sifted and extracted using diethyl ether anhydrous. Androgen content was analysed in the faecal extracts by radioimmunoassay (RIA) using a previously validated assay for bison (Mooring et al. 2004b), with two procedural differences. First, 25  $\mu\text{l}$  or 100  $\mu\text{l}$  of a 1:100 dilution (ethanolic faecal extract:phosphate buffered saline pH 7.0) were used in the assay. Second, the interassay coefficients of variation (%SD/mean,  $N = 6$ ) were 7.9%, based on duplicates of a standard with an immunoreactive content that yielded

a percentage of total binding (%B/BO) > 60%, and 6.4%, based on duplicates of a standard with an immunoreactive content that yielded a %B/BO > 25%. Details of the procedure are reported in Mooring et al. (2004b).

## Statistical Analysis

Data were analysed using the SPSS 11.0 statistical package for Windows (Norusis 2002). We used nonparametric statistical tests for analysis of behavioural measures (Martin & Bateson 1993). Tests included one-sample analyses of variance (ANOVAs) with Scheffé multiple comparisons on ranked data, Mann–Whitney *U* tests and Spearman rank-order tests (Siegel & Castellan 1988). The level of significance was set at 0.05, and all tests were two tailed.

## RESULTS

### Grooming Rates

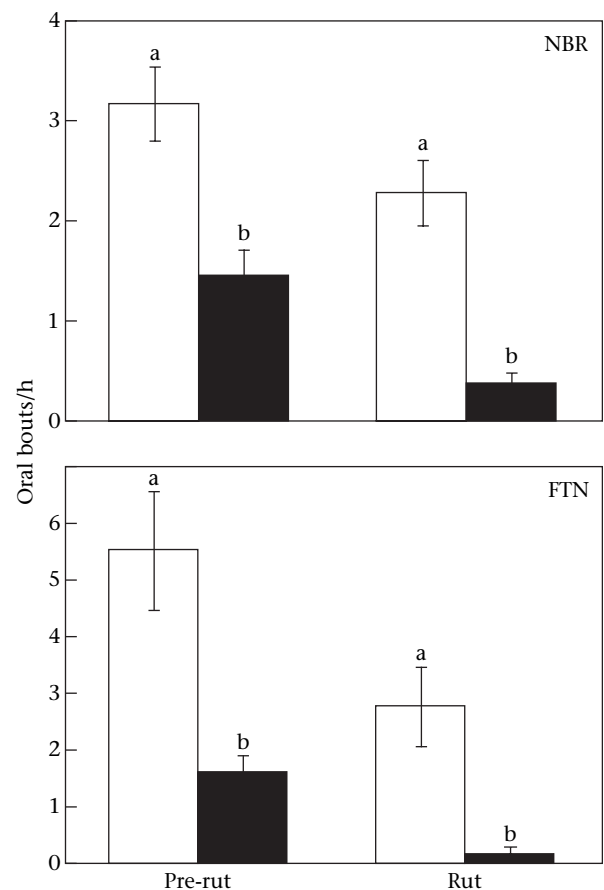
During the time of sexual segregation (pre-rut), adult female and male bison at NBR showed a significant difference in oral-grooming rate (Mann–Whitney *U* test: oral bouts:  $Z = 3.48$ ,  $N_{\text{female}} = 144$ ,  $N_{\text{male}} = 99$ ,  $P = 0.0001$ ; oral episodes:  $Z = 3.23$ ,  $P = 0.001$ ), with females oral grooming about twice as frequently as males (Figs 1, 2). There was no difference in scratch-grooming rate between the sexes (scratch bouts:  $Z = 1.45$ ,  $P = 0.15$ ; scratch episodes:  $Z = 1.41$ ,  $P = 0.16$ ). Data collected at FTN confirmed the above results for known males and females (Figs 1, 2). Oral-grooming rates of females at FTN were three to five times greater than those of males (Mann–Whitney *U* test: oral bouts:  $Z = 3.01$ ,  $N_{\text{female}} = 45$ ,  $N_{\text{male}} = 40$ ,  $P = 0.003$ ; oral episodes:  $Z = 2.60$ ,  $P = 0.009$ ).

Analysis of variance revealed a significant difference in pre-rut oral-grooming rates among adult females, adult males and subadult males at NBR (Table 1), but no difference in scratch-grooming rates ( $P > 0.27$ ). According to Scheffé multiple comparisons, the rate at which subadult males delivered oral-grooming bouts was greater than that of adult males, but no different from that of females. A similar pattern was seen for oral-grooming episodes, with subadult males oral grooming more frequently than adult males but at a rate no different from that of adult females (Table 1).

During the time of sexual aggregation (rut) at NBR, there was a significant difference in oral- and scratch-grooming rates (grooms/h) between adult female and adult male bison in tending pairs (Mann–Whitney *U* test: oral bouts:  $Z = 5.22$ ,  $N_{\text{female}} = 144$ ,  $N_{\text{male}} = 99$ ,  $P = 0.0001$ ; oral episodes:  $Z = 5.00$ ,  $P = 0.0001$ ; scratch bouts:  $Z = 2.74$ ,  $P = 0.006$ ; scratch episodes:  $Z = 2.74$ ,  $P = 0.006$ ), with females oral grooming five to six times as frequently as males, compared with two times as frequently during pre-rut (Figs 1, 2). Breeding males at NBR during the rut groomed much less than males during pre-rut. Oral-grooming rates of tending males were 26–40% of pre-rut rates (Mann–Whitney *U* test: oral bouts:  $Z = 3.13$ ,  $N_{\text{pre-rut}} = 99$ ,  $N_{\text{tending}} = 99$ ,  $P = 0.002$ ;

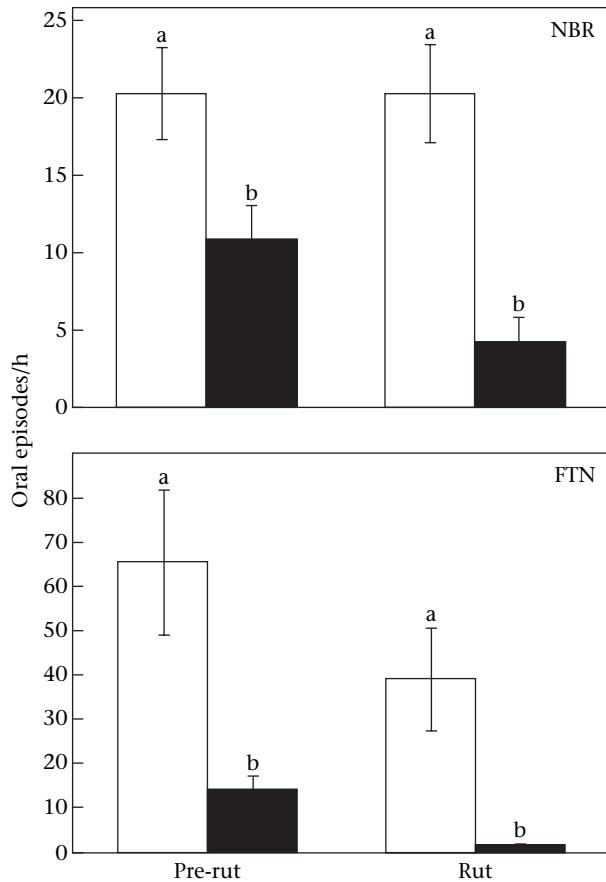
oral episodes:  $Z = 2.97$ ,  $P = 0.003$ ), while scratch-grooming rates of tending males were only 2–6% of pre-rut rates (scratch bouts:  $Z = 3.16$ ,  $P = 0.002$ ; scratch episodes:  $Z = 3.18$ ,  $P = 0.001$ ). In contrast, there were no significant differences in grooming rates between pre-rut females and tended females during the rut (oral bouts:  $Z = 1.07$ ,  $P = 0.29$ ; oral episodes:  $Z = 0.34$ ,  $P = 0.74$ ; scratch bouts:  $Z = 0.95$ ,  $P = 0.34$ ; scratch episodes:  $Z = 0.80$ ,  $P = 0.42$ ). Subadult males were not examined during the rut.

Tended females at FTN also oral-groomed at higher rates than did tending males during the rut (Mann–Whitney *U* test: oral bouts:  $Z = 5.08$ ,  $N_{\text{female}} = 69$ ,  $N_{\text{male}} = 47$ ,  $P = 0.0001$ ; oral episodes:  $Z = 5.13$ ,  $P = 0.0001$ ), with females grooming 16–39 times more frequently than males, compared with three to five times higher during pre-rut (Figs 1, 2). Tending males during the rut oral-groomed at 7–11% of pre-rut rates (oral bouts:  $Z = 4.06$ ,  $N_{\text{pre-rut}} = 40$ ,  $N_{\text{rut}} = 47$ ,  $P = 0.0001$ ; oral episodes:  $Z = 4.08$ ,  $P = 0.0001$ ), while rates of scratch grooming during the rut were only 4% of pre-rut rates (scratch bouts:  $Z = 3.60$ ,  $N_{\text{pre-rut}} = 40$ ,  $N_{\text{rut}} = 47$ ,  $P = 0.0001$ ; scratch episodes:  $Z = 3.56$ ,  $P = 0.0001$ ). However, unlike at NBR, female rates of oral grooming during rut at FTN were less



**Figure 1.** Oral-grooming bouts performed per h by adult female (□) and adult male (■) bison at National Bison Range in 2002 (NBR) and Fort Niobrara in 2004 (FTN) during the pre-rut and rut. Different letters indicate a significant difference ( $P < 0.003$ ) by the Mann–Whitney *U* test.





**Figure 2.** Oral-grooming episodes performed per h by adult female (□) and adult male (■) bison at National Bison Range in 2002 (NBR) and Fort Niobrara in 2004 (FTN) during the pre-rut and rut. Different letters indicate a significant difference ( $P < 0.01$ ) by the Mann–Whitney  $U$  test.

than pre-rut rates (oral bouts:  $Z = 2.77$ ,  $N_{\text{pre-rut}} = 45$ ,  $N_{\text{rut}} = 69$ ,  $P = 0.006$ ; oral episodes:  $Z = 1.90$ ,  $P = 0.057$ ).

### Activity Budgets

We report primarily on the activity budgets of NBR bison, which had the largest sample sizes. During pre-rut, when males and females were sexually segregated, activity budgets differed little between the sexes. Females spent

**Table 1.** Mean oral-grooming rates of adult male and female and subadult male bison during pre-rut at National Bison Range, Montana during 2002, and statistics for ranked ANOVA

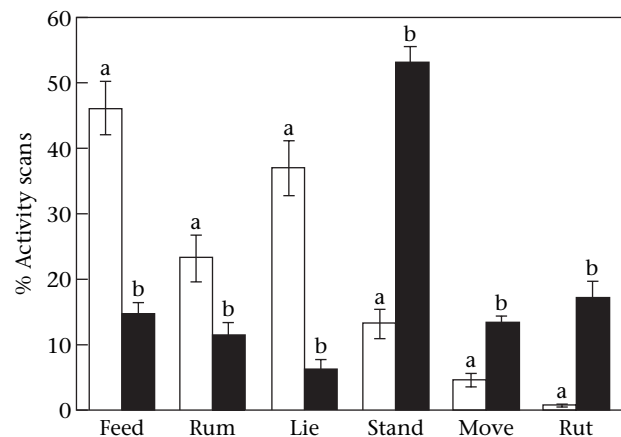
	Adult females	Adult males	Subadult males	$F_{2,293}$	$P$
Oral bouts	3.17 <sup>a</sup>	1.45 <sup>b</sup>	3.79 <sup>a</sup>	12.3	0.0001
Oral episodes	20.35 <sup>a</sup>	10.79 <sup>b</sup>	22.58 <sup>a</sup>	11.2	0.0001
Scratch bouts	0.69 <sup>a</sup>	0.48 <sup>a</sup>	0.40 <sup>a</sup>	1.3	0.27
Scratch episodes	5.02 <sup>a</sup>	4.91 <sup>a</sup>	3.00 <sup>a</sup>	1.3	0.29

Different superscript letters indicate a significant difference by Scheffé multiple comparisons.

more time standing up than males, and males spent more time wallowing than females (Mann–Whitney  $U$  test: stand:  $Z = 2.73$ ,  $N_{\text{female}} = 144$ ,  $N_{\text{male}} = 99$ ,  $P = 0.006$ ; wallow:  $Z = 2.94$ ,  $P = 0.003$ ), otherwise there were no differences between the sexes. During rut, when males and females were aggregated in mixed herds, tending males spent more time engaged in rutting behaviours, standing and movement, and less time feeding, compared with tended females (all rutting behaviours:  $Z = 9.06$ ,  $N_{\text{female}} = 119$ ,  $N_{\text{male}} = 99$ ,  $P = 0.0001$ ; stand:  $Z = 4.86$ ,  $P = 0.0001$ ; walk:  $Z = 2.72$ ,  $P = 0.006$ ; move (walk and run):  $Z = 2.66$ ,  $P = 0.008$ ; feed:  $Z = 3.96$ ,  $P = 0.0001$ ). In terms of specific rutting activities, tending males spent more time performing flehmen, wallowing, pawing, bellowing, tending and mounting than did tended females (flehmen:  $Z = 3.90$ ,  $P = 0.0001$ ; wallow:  $Z = 3.06$ ,  $P = 0.002$ ; paw:  $Z = 4.69$ ,  $P = 0.0001$ ; bellow:  $Z = 7.38$ ,  $P = 0.0001$ ; tend:  $Z = 2.94$ ,  $P = 0.003$ ; mount:  $Z = 2.72$ ,  $P = 0.007$ ).

Comparing activity rates (percentage of scans) of males during pre-rut and rut (Fig. 3), tending males during the rut spent less time feeding, ruminating and lying down (Mann–Whitney  $U$  test: feed:  $Z = 4.57$ ,  $N_{\text{pre-rut}} = 99$ ,  $N_{\text{rut}} = 99$ ,  $P = 0.0001$ ; ruminate:  $Z = 2.02$ ,  $P = 0.04$ ; lie:  $Z = 6.38$ ,  $P = 0.0001$ ), and more time standing, moving (walking and running) and rutting than did pre-rut males (stand:  $Z = 9.74$ ,  $P = 0.0001$ ; move:  $Z = 7.18$ ,  $P = 0.0001$ ; rut:  $Z = 7.41$ ,  $P = 0.0001$ , which includes flehmen:  $Z = 3.56$ ,  $P = 0.0001$ ; paw:  $Z = 4.30$ ,  $P = 0.0001$ ; bellow:  $Z = 6.79$ ,  $P = 0.0001$ ; tend:  $Z = 2.69$ ,  $P = 0.007$ ; mount:  $Z = 2.48$ ,  $P = 0.01$ ). Focal activity scans also showed that tending males during the rut spent less time oral grooming compared with pre-rut males ( $Z = 2.44$ ,  $P = 0.015$ ).

We examined the activity budgets of tending males (actively guarding females), nontending males in mixed herds (some associated with tending pairs) and males in bull groups (and therefore not engaged in breeding). Tending, nontending and bull group males differed significantly in the time that they spend rutting, standing, moving (walking or running) and lying down (ranked

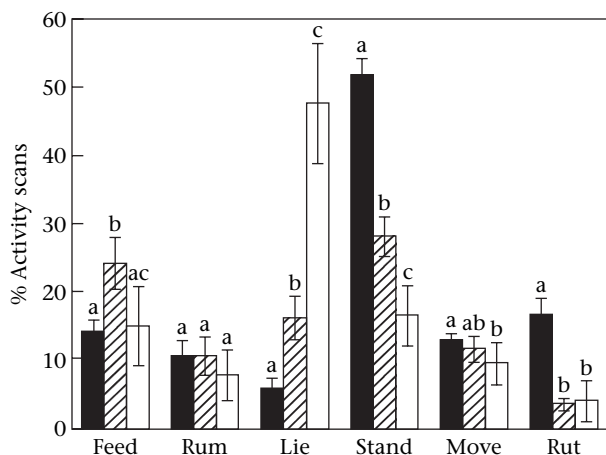


**Figure 3.** Activity budgets (percentage of focal activity scans) of bison males during pre-rut (□) and rut (■) at National Bison Range (NBR) in 2002. Different letters indicate a significant difference ( $P < 0.04$ ) by the Mann–Whitney  $U$  test. Rum = ruminate.

ANOVA: all rutting behaviours:  $N_{\text{tend}} = 99$ ,  $N_{\text{nontend}} = 52$ ,  $N_{\text{bull group}} = 24$ ,  $F_{2,172} = 9.62$ ,  $P = 0.0001$ ; stand:  $F_{2,172} = 35.08$ ,  $P = 0.0001$ ; move:  $F_{2,172} = 3.29$ ,  $P = 0.04$ ; lie:  $F_{2,172} = 23.63$ ,  $P = 0.0001$ ; Fig. 4). According to Scheffé multiple comparisons, tending males spent more time engaged in rutting behaviours ( $P < 0.01$ ) and standing ( $P = 0.0001$ ) and less time lying down ( $P < 0.001$ ) compared with nontending or bull group males. Tending males also spent more time moving than bull group males ( $P_{\text{tend-bull}} = 0.05$ ), but not more than nontending males ( $P_{\text{tend-nontend}} = 0.51$ ). In terms of specific rutting behaviour, tending males spent more time performing flehmen than bull group males, but not more than nontending males (Scheffé multiple comparisons:  $P_{\text{tend-bull}} = 0.03$ ,  $P_{\text{tend-nontend}} = 0.18$ ), and tending males bellowed and pawed ( $P < 0.001$ ) more than nontending and bull group males (Fig. 4; ranked ANOVA: flehmen:  $F_{2,172} = 4.00$ ,  $P = 0.02$ ; bellow:  $F_{2,172} = 15.10$ ,  $P = 0.0001$ ; paw:  $F_{2,172} = 2.97$ ,  $P = 0.05$ ).

Despite these differences in activity budgets, there were no differences in grooming rates between tending, nontending and bull group males (ranked ANOVA: oral bouts:  $F_{2,172} = 1.57$ ,  $N_{\text{tend}} = 99$ ,  $N_{\text{nontend}} = 52$ ,  $N_{\text{bull group}} = 24$ ,  $P = 0.21$ ; oral episodes:  $F_{2,172} = 1.50$ ,  $P = 0.23$ ; scratch bouts:  $F_{2,172} = 2.09$ ,  $P = 0.13$ ; scratch episodes:  $F_{2,172} = 2.10$ ,  $P = 0.13$ ). In addition, there were no significant differences between males in time spent grooming according to focal activity scans (oral grooming:  $F_{2,172} = 1.31$ ,  $P = 0.27$ ; scratch grooming:  $F_{2,172} = 1.06$ ,  $P = 0.35$ ).

Activity budgets of bison at FTN were similar to those at NBR, despite smaller sample sizes. Tending males during the rut spent much more time engaged in rutting behaviours, bellowing and standing (Mann–Whitney  $U$  test: all rutting behaviours:  $Z = 5.74$ ,  $N_{\text{pre-rut}} = 36$ ,  $N_{\text{rut}} = 18$ ,  $P = 0.0001$ ; bellow:  $Z = 6.56$ ,  $P = 0.0001$ ; stand:  $Z = 2.87$ ,  $P = 0.004$ ) and less time lying down (lie:



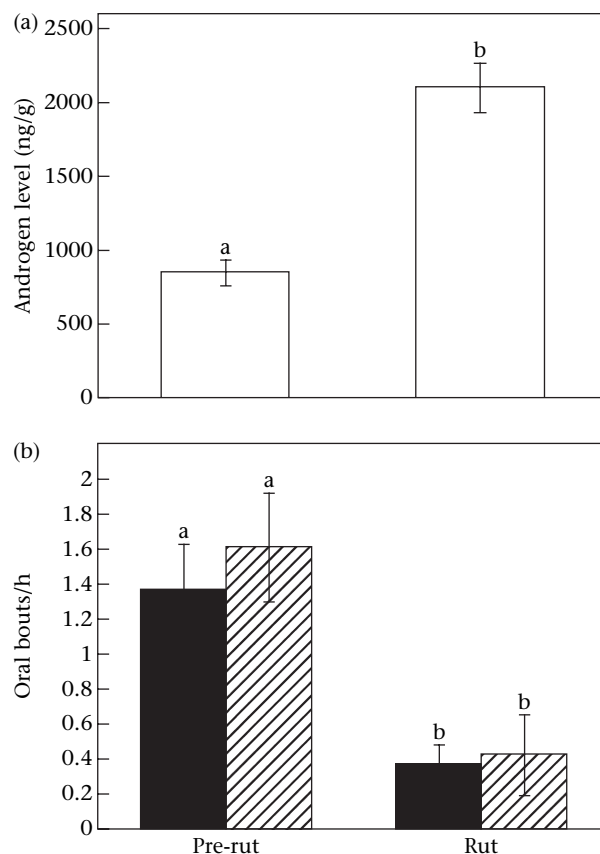
**Figure 4.** Activity budgets (percentage of focal activity scans) of bison males during the rut at National Bison Range in 2002: tending males (■), nontending males (▨) and males in bull groups (□). Tending males were actively guarding females, nontending males participated in some rutting activities, and males in single-sex bull groups did not engage in rutting behaviour. Different letters indicate a significant difference ( $P < 0.04$ ) by ranked ANOVA. Rum = ruminate.

$Z = 3.41$ ,  $P = 0.001$ ) compared with pre-rut males. There was no difference in grooming rates among tending and nontending males during the rut (oral bouts:  $Z = 1.58$ ,  $P = 0.12$ ; oral episodes:  $Z = 1.59$ ,  $P = 0.11$ ; scratch bouts:  $Z = 0.75$ ,  $P = 0.45$ ; scratch episodes:  $Z = 0.75$ ,  $P = 0.45$ ).

## Androgens and Grooming Rate

Males at FTN during 2004 had significantly higher levels of androgens during the rut compared with pre-rut (Fig. 5). The mean ( $\pm$ SE) androgen level of pre-rut males ( $845 \pm 87$  ng/g) more than doubled during the rut ( $2096 \pm 167$  ng/g), and this increase was significant (Mann–Whitney  $U$  test:  $Z = 5.3$ ,  $N_{\text{pre-rut}} = 24$ ,  $N_{\text{rut}} = 31$ ,  $P = 0.0001$ ). During the rut, mean androgen level of tending males ( $2574 \pm 196$  ng/g) was nearly double that of nontending males ( $1433 \pm 168$  ng/g;  $Z = 3.7$ ,  $N_{\text{tend}} = 18$ ,  $N_{\text{nontend}} = 13$ ,  $P = 0.0001$ ).

Oral-grooming and scratch-grooming rates of FTN males (pre-rut and rut, with no individuals duplicated) were negatively associated with androgen level, but only the correlation between oral grooming and androgen level was significant (Spearman rank-order correlation: oral



**Figure 5.** (a) Androgen levels of bison males at Fort Niobrara during 2004 and (b) oral-grooming bouts performed per h by males at National Bison Range in 2002 (■) and Fort Niobrara in 2004 (▨) during the pre-rut and rut. Grooming rates during the rut refer to tending males. Different letters indicate a significant difference ( $P < 0.002$ ) by the Mann–Whitney  $U$  test.

bouts:  $r_s = -0.35$ ,  $N = 55$ ,  $P = 0.008$ ; oral episodes:  $r_s = -0.36$ ,  $N = 55$ ,  $P = 0.008$ ; scratch bouts and episodes:  $r_s = -0.26$ ,  $N = 55$ ,  $P = 0.06$ ). Although oral-grooming rate was negatively correlated with androgens for the subset of pre-rut males (oral bouts:  $r_s = -0.46$ ,  $N = 24$ ,  $P = 0.02$ ; oral episodes:  $r_s = -0.45$ ,  $N = 24$ ,  $P = 0.03$ ), there was no significant correlation for the subset of tending males during rut (oral bouts and episodes:  $r_s = -0.03$ ,  $N = 35$ ,  $P = 0.86$ ) because the extremely low rate of oral grooming performed by tending males (mean  $\pm$  SE:  $0.06 \pm 0.24$  oral bouts/h;  $0.4 \pm 1.4$  oral episodes/h) resulted in virtually no variation in grooming rate.

## DISCUSSION

### Body Size and Vigilance Effects

We examined the predictions of the programmed grooming hypothesis for sexually dimorphic grooming, focusing on the role of body size, activity budget and androgen levels of bison bulls. Our results support the predictions of both the body size and vigilance principles and demonstrate that these effects operate independently and additively in bison. Females groomed significantly more than males during both pre-rut and rut, whereas tending males during the rut groomed significantly less than pre-rut males. For example, at NBR, oral-grooming rates of females were about twice those of adult males during pre-rut (as predicted by the body size principle), but during the rut, the rate of female oral grooming was five to six times greater than that of tending males that were actively guarding females (as predicted by the vigilance principle). Similarly, at FTN, female grooming rate was three to five times higher than that of males during pre-rut and 16–40 times higher than that of males during the rut. Oral-grooming rates of tending males during rut were less (26–40%) than those of pre-rut males at both sites, as the vigilance principle predicts. During pre-rut, the oral-grooming rate of subadult males, of similar size to females, was no different from female grooming rate but was higher than that of larger adult males, as predicted by the body size principle.

### Role of Activity Budgets

Reduction of oral-grooming rates in breeding males during the rut could be due to two proximate mechanisms. Either the performance of rutting behaviours (e.g. tending, pawing, wallowing, bellowing, fighting) is so demanding that males do not have much time available to groom (activity budget conflicts), or elevated levels of circulating androgens are responsible for a physiological suppression of oral grooming. Regarding the first explanation, an observational field study (Mooring et al. 1996b) revealed that solitary territorial male impala without females on their territory (and thus not engaged in breeding behaviour) oral-groomed at the same low rate as territorial males in the presence of females (who were engaged in breeding activities). This indicates that suppression of oral grooming in impala males was not due to the

conflicting activities of breeding. In the present study of NBR bison, nontending males and males in bull groups spent significantly less time engaged in rutting activities and more time lying down compared with tending males, yet all males oral-groomed at the same low rate. This effect was especially dramatic for bull group males, for which no females were present to require vigilance and nearly 50% of the activity budget was spent lying down. This indicates that the vigilance-mediated reduction of oral grooming by bison males during the rut is not the result of conflicting activities, and points to a physiological suppression of grooming.

### Role of Androgens

Previous experimental work has indicated that elevated androgens in breeding male ungulates are associated with a decline in oral-grooming rate, suggesting a causal relationship in which increased levels of androgens act on the grooming control centre of the CNS to down-regulate oral-grooming rate. In dairy goats, experimental castration resulted in a significant increase in oral-grooming rates of males over 2–8 weeks, albeit with individual variation in grooming response (Mooring et al. 1998). Mean oral-grooming rate of castrated goats was elevated three-fold over that of intact males, with individual increases ranging from two to 14 times above intact males (Mooring et al. 1998). In dwarf Shiba goats, long-term castrated males oral-groomed at higher rates than did intact males (Kakuma et al. 2003). Subcutaneous implantation of testosterone in the castrated males suppressed oral grooming to the same low level of intact males within 2 weeks, whereas removal of the implants resulted in elevation of oral grooming to baseline rates within a week (Kakuma et al. 2003). These studies support the prediction that testosterone mediates the suppression of oral grooming. Testosterone could modulate grooming either by direct action on neurons, through intermediary metabolites such as oestrogen or dihydrotestosterone, or by influencing other neurotransmitters, such as vasopressin, which can alter grooming rates (Meisenberg 1988).

In this study, both tending and nontending males experienced an increase in faecal androgens between pre-rut and rut, although tending males had higher levels of androgens compared with nontending males; this was also found in a previous study of bison (Mooring et al. 2004b). Elevated androgens during rut appear to have suppressed male grooming to such a low rate that no significant difference could be detected between tending and nontending males. Our study showed a negative correlation between individual androgen levels and the oral-grooming rate of bison males, indicating that bulls with elevated androgens groomed less.

## Conclusion

Sexual selection favours morphological, physiological and behavioural traits that improve competition for mates, such as large size, weaponry and threat displays (Darwin 1859, 1871; Andersson 1994). These traits are

sexually dimorphic and are often under the influence of androgens. Sexually dimorphic grooming has coevolved with sexual body size dimorphism, partly as the result of sexual selection for intense male–male competition (Mooring et al. 2002, 2004a). Mooring et al. (2004a) concluded that sexual selection has simultaneously selected for dimorphism in features that directly influence male competitive ability (e.g. body size) and features that indirectly influence mating success by improving vigilance for oestrous females and rival males (e.g. reduced grooming).

In bison, both the body size and vigilance effects of the programmed grooming model work independently and additively to produce sexually dimorphic oral grooming. The reduced oral-grooming rate of males relative to females outside of the rut can be explained primarily by body size differences (we cannot rule out a marginal effect from androgen differences). The additional decrement in grooming rate by males during the rut is most likely due to the proximate action of elevated androgens (including testosterone) in activating a physiological suppression of programmed grooming. Our results do not support the action of conflicting activity budgets (increased rutting activities) in reducing oral-grooming rates. The androgen-mediated suppression of grooming observed in this study suggests that sexually dimorphic grooming in rutting bison is guided ultimately by sexual selection for highly competitive males that are constantly vigilant for opportunities to maximize their reproductive success.

### Acknowledgments

We thank National Bison Range and Fort Niobrara national wildlife refuges for permission to study their bison herds and for providing refuge housing and use of vehicles during our study. We are particularly grateful to Dave Wiseman, Lindy Garner and Lynn Verlanic at National Bison Range, and Royce Huber, Bernie Petersen and Kathy McPeak at Fort Niobrara for their support and assistance. We also thank Michael Dillenbeck, Desiree Boyle, Matt Herrick and Nikki Beckley for their assistance in collecting faecal samples at Fort Niobrara in 2004. Funding for this study was provided by the Research Associates, and by Research and Special Project grants, a Dean's Discretionary grant, an Alumni award, and a Wesleyan Center scholarship from Point Loma Nazarene University. The comments of John Byers and two anonymous referees helped to improve the manuscript.

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