



Stress hormones and social behavior of wolves in Yellowstone National Park
by Jennifer Leigh Sands

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Biological Sciences
Montana State University
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Abstract:

Animals living within social hierarchies potentially deal with chronic stressors due to their involvement in agonistic behavioral interactions and aggressive contests for dominance. Research on other species, mostly conducted in captivity, suggests that social stress falls on subordinates, provoking a physiological stress response, mediated in part by increased secretion of glucocorticoids (GCs). Chronically elevated GCs can suppress reproduction (among other harmful effects), so it is logical to hypothesize that reproductive suppression among subordinate cooperative breeders is a consequence of social stress. However, our research, consistent with a pattern emerging from field research on cooperatively breeding animals, suggests that this is not the mechanism by which subordinates are reproductively suppressed. In cooperative breeders, where GC levels differ according to rank it is the dominant individuals whose levels are elevated. This study employed non-invasive fecal sampling and behavioral observation methods to determine relationships among social dominance, aggression, reproduction and stress hormone levels in the Druid Peak, Rose Creek and Leopold packs of wolves in Yellowstone National Park. Higher-ranking wolves had significantly higher concentrations of GCs than subordinates. While rank was a good predictor of stress hormone levels, the rates of aggressive and agonistic behaviors were not strongly correlated with GC levels. While we know that the GC levels of dominant wolves are elevated, we are still left with questions concerning what aspects of dominance are stressful. Elevated GC levels in higher-ranking animals indicate that there are some hidden physiological costs to dominance.

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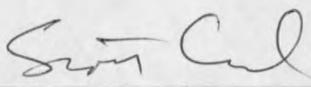
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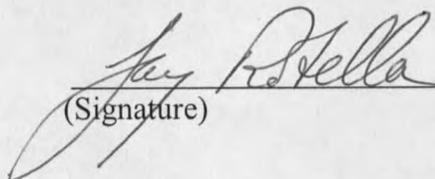


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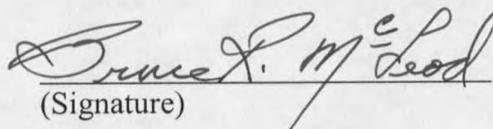


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ABSTRACT

Animals living within social hierarchies potentially deal with chronic stressors due to their involvement in agonistic behavioral interactions and aggressive contests for dominance. Research on other species, mostly conducted in captivity, suggests that social stress falls on subordinates, provoking a physiological stress response, mediated in part by increased secretion of glucocorticoids (GCs). Chronically elevated GCs can suppress reproduction (among other harmful effects), so it is logical to hypothesize that reproductive suppression among subordinate cooperative breeders is a consequence of social stress. However, our research, consistent with a pattern emerging from field research on cooperatively breeding animals, suggests that this is not the mechanism by which subordinates are reproductively suppressed. In cooperative breeders, where GC levels differ according to rank it is the dominant individuals whose levels are elevated. This study employed non-invasive fecal sampling and behavioral observation methods to determine relationships among social dominance, aggression, reproduction and stress hormone levels in the Druid Peak, Rose Creek and Leopold packs of wolves in Yellowstone National Park. Higher-ranking wolves had significantly higher concentrations of GCs than subordinates. While rank was a good predictor of stress hormone levels, the rates of aggressive and agonistic behaviors were not strongly correlated with GC levels. While we know that the GC levels of dominant wolves are elevated, we are still left with questions concerning what aspects of dominance are stressful. Elevated GC levels in higher-ranking animals indicate that there are some hidden physiological costs to dominance.

INTRODUCTION

Animals face a variety of stressors in the wild including food availability, unpredictable climate fluctuations, human disturbance, aggression and social status. Though the stressors may be highly variable, the physiological response to stress is similar. Within seconds from brain recognition of a stressor, the adrenal gland secretes the hormone adrenaline. Blood pressure, heart rate and breathing rate escalate rapidly as energy is quickly mobilized and transported for immediate use. Within minutes, the adrenal cortex secretes glucocorticoids (GCs), another class of hormones central to the stress response. Glucocorticoids also mobilize necessary energy by altering metabolic pathways for the production of ATP, but just as importantly, GCs divert energy from physiological processes not required for immediate survival such as digestion, growth, immune function and reproduction (Sapolsky 1992a; Munck et al.1984). The stress response can be beneficial to an animal in the short term, but if activated consistently and for prolonged periods of time, may become harmful. If GCs are chronically elevated, the inhibition of anabolic processes such as growth, digestion and reproductive hormone secretion can result in reproductive failure, decreased resistance to disease, brain atrophy and shorter life-span (Sapolsky 1992a; Pottinger 1999).

Animals that live in social hierarchies potentially deal with chronic stressors due to their involvement in agonistic behavioral interactions and aggressive contests for dominance (Wingfield et al.1991; Sapolsky 1992b). Investigations into the relationship between GC levels, aggression and social status have been conducted since the 1950's (Davis & Christian, 1957). Although differing results have been reported in the last 50

years, the results of early research on social stress have strongly influenced the pervasive view that subordination is stressful.

Early endocrine research, focused on rodents (Louch & Higginbotham 1967; Bronson 1973; Bronson & Eleftheriou 1964) and primates (Manogue 1975), indicated that social subordinates commonly have chronically elevated GC levels. These influential "arena" studies were conducted with captive individuals who were housed singly, sometimes screened for their aggressiveness (Blanchard et al. 1995), then grouped or paired with unfamiliar animals. The fighting that ensued was then observed and scored on the basis of biting, injury and weight loss. The individual who had initiated more bites, had fewer wounds and retained his weight was considered the winner and consequently, the "dominant" animal. Though both animals had elevated GC levels due to the intensity of aggression, the loser or "subordinate" animal consistently had higher GC levels than the winning, or "dominant" animal. Results from these captive winner/loser studies became the basis of a subsequent theory concerning the mechanism of reproductive suppression in cooperatively breeding species. Since chronic GC elevation can decrease sex steroid secretion and inhibit reproduction (Welsh & Johnson 1981; Moberg 1985), several authors (Wingfield 1988; Blanchard et al. 1995) linked the elevated GCs secreted in captive "subordinate" animals to the absence of reproduction in socially subordinate cooperative breeders in the wild. This common argument, that the 'stress of subordination' might underlie reproductive suppression of subordinates is also known as the 'psychological castration' hypothesis (Brown 1978; Reyer 1986).

Though a logical progression of thoughts, these behavioral and endocrine data were extrapolated from conditions of captivity and applied to natural assemblages of animals living in the wild. Much of the work in captivity was conducted with domesticated animals derived from solitary ancestral species, rather than with species that naturally live in stable social groups. This early captive research elucidated the relationship between aggression and GC levels, but the endocrine correlates of winning fights may bear little resemblance to the correlates of dominance in the wild (Creel et al. 1996b). Animals that live in permanent stable social groups have evolved stylized behaviors and postures (e.g. tail position, ear position, snarl) to assert and maintain their established relationships and rank order (Kleiman 1967; Schenkel 1967). These behaviors serve to avoid the costs and risks associated with fighting and intense aggression (Sapolsky 1992a). Also, subordinate animals in wild populations can disperse to avoid further aggression, while this is not an option for animals in captivity. These considerations bring to light the importance of studying the relationship between GCs, aggression and social status outside the confines of captivity with species living in naturally formed permanent social groups.

Field studies of behavioral endocrinology in social birds and mammals suggest that the traditional view of stress as a cost of subordination, and its link to reproductive suppression may be too simple. Data from studies of wild populations indicate that the relationship between rank and GC levels varies among species and within species, as male and female patterns sometimes can be dissimilar. In Olive baboons (*Papio anubis*) subordinates are hypercortisolemic when their hierarchy is stable, otherwise the dominant

individuals' GC levels are elevated (Sapolsky 1992b). In Harris' hawks (*Parabuteo unicinctus*: Mays, et al. 1991), white-browed sparrow-weavers (*Plocepasser mahali*: Wingfield et al. 1991), male dwarf mongooses (*Helogale parvula*: Creel et al. 1992) and male Florida scrub jays (*Aphelocoma coerulescens*: Schoech et al. 1991) basal GC levels are not affected by dominance. Dominant female dwarf mongooses and Florida scrub jays had significantly higher basal GC levels than subordinates (Schoech et al. 1991; Creel et al. 1992), as did both male and female dominant African wild dogs (*Lycaon pictus*: Creel et al. 1996a). A pattern is emerging from these more recent field studies: if there is a difference according to rank, more often it is the dominants, not subordinates who are socially stressed.

Studies of several cooperatively breeding species have also addressed the association between GCs and reproduction, and found that dominant individuals with elevated GC levels maintained normal patterns of reproductive hormone secretion while subordinates did not, despite low GC levels (e.g. common marmosets (*Callithrix jacchus*): Abbott et al. 1981; cotton-top tamarins (*Saguinus oedipus*): Ziegler et al. 1995; and dwarf mongooses: Creel et al. 1992). Sex steroid secretion of cooperative breeders is affected by rank in a number of species studied, but the validity of the "psychological castration" explanation --that reproductive inhibition of subordinates is stress induced is questionable. Data from more species on the association between stress physiology, dominance and reproduction in the wild will resolve whether or not stress affects reproductive function of subordinate animals.

It is now possible to measure GC levels in a non-invasive manner, using fecal hormone radioimmunoassays (Creel et al. 1996a; Wasser et al. 1997). There are a number of reasons that fecal hormone measurement is preferable to blood sampling when measuring baseline GC levels. Fecal samples can be collected once an animal moves away (after defecating) without having to capture, immobilize or restrain the animal to obtain the sample, which can itself be a stressor. Long-term stressors, such as psychological stress related to social status, will show their effects as a change in basal GC values (the levels when an animal is *not* responding to a short-term stressor). Circulating GC levels will increase within minutes of handling, interfering with the baseline measurements that are sought, unless blood sampling occurs quickly and consistently after first disturbing the animal (Creel 2001). Measurements of GCs using fecal samples are more representative of an individual's basal GC levels since GCs are pooled over a number of hours in a fecal sample, integrating changes in hormone secretion over the time that the defecation accumulated; whereas a measurement from blood is a 'snapshot' of GC levels at the given instant it is drawn and may not reflect the true baseline (Monfort et al. 1993). Fecal sampling also has some weaknesses compared to blood sampling. The measurements can be complicated by individual variation in the diet and the variance of hormone profiles may be higher than profiles from blood sampling. Consequently, more samples may be required in non-invasive studies and methods to control for variation in diet must be in place.

The social organization of wolves is well-suited to the study of social stress in a cooperative breeding carnivore. Typical of group living canids, wolves live in permanent

social groups composed primarily of closely related individuals who live within a stable dominance hierarchy. Though subordinates sometimes reproduce, normally the alpha male and female are the only animals assured of breeding opportunities. We studied a population of gray wolves (*Canis lupus*), reintroduced into Yellowstone National Park in 1995 (Fritts et al 1997). Because the wolves have full protection inside the park, they are less wary of humans than most other wolf populations in the world. The terrain where the study was conducted is dominated by valleys where line of sight visibility commonly exceeds five kilometers, reliably providing viewing opportunities of wolves. As part of ongoing long-term research, the National Park Service maintains radio-collars on approximately 35% of the population, facilitating collection of data on age, social status, survival and reproduction. For all of the above reasons the wolves can be tracked, are easily observed and individually identifiable, providing an unique opportunity to study a species that is commonly difficult to observe in the wild.

The goals of this study are to determine whether dominant or subordinate wolves are paying the cost of social stress. As mentioned above it has been widely argued that subordination is stressful. Subordinate cooperative breeders do not commonly breed, they help raise offspring that are not their own and are often the target of aggression from other pack members; while dominant animals are seldom aggressively challenged for their position and generally have greater reproductive success than subordinates. From an evolutionary perspective it seems that dominants reap most of the benefits from the social organization, but recent studies of cooperative breeders in the wild suggest that dominants, not subordinates have elevated GC levels, indicating that there are fitness

costs associated with dominance. Though relatively few such studies of social stress and stress physiology have been conducted in the wild, the results dispute data from captivity (mostly from species that do not breed cooperatively), which suggest that social stress falls on subordinates. Is the pattern of social stress in wolves similar to these other studies of cooperative breeders in the wild? We addressed this question through behavioral observation and collection of fecal samples from three packs of wolves in YNP. We 1) identified the dominance structure of these packs, 2) non-invasively collected fecal samples from these wolves, 3) assayed the samples for GC concentrations, and 4) explored the correlations between GC levels and rank, aggression, rates of behaviors, and reproduction. Establishing whether social stress is a cost of dominance or subordination pertains to significant questions in behavioral ecology concerning the evolution of social systems and why social subordinates tolerate reproductive suppression.

METHODS

Study Population

We studied the Druid Peak, Rose Creek and Leopold packs between January 1999 and July 2000 in Yellowstone National Park (YNP). The open sage/grassland valleys that dominated our study area allowed for observation along most of the valley floor and on unforested sections of slopes that surrounded the valleys. The Northeast Entrance road is maintained year-round and intersects all three packs' home ranges, greatly improving accessibility. The elevation of the study area ranged from 1600 to 2900m. For the years of the study there was an average yearly low temperature of -8°C and a high of 12°C . The average snow depth was 19.6 cm and the average snow water equivalent (SWE), the equivalent amount of water contained in a column of snow, was 6.9 cm for the time snow was on the ground between November and May 1999 and 2000.

The population of wolves in the greater Yellowstone area was established by reintroducing a total of 31 wolves into YNP in 1995 and 1996 (Fritts et al. 1997). During the years of this study, the population was estimated to range between 112 and 118 individuals. (Smith et al. 1999; Smith et al. 2000). We selected the Druid Peak, Rose Creek and Leopold packs as our focal packs because they were the most easily observed, and other scientists studying the same packs could often provide sightings. In January of 1999 there were 7, 22 and 13 members of the Druid Peak, Rose Creek and Leopold packs, respectively. In January of 2000, there were 8, 16, and 11 members, respectively. During the study, a minimum of four wolves in each pack wore radio-collars.

Behavioral Observations

Packs were located using radio telemetry and observed opportunistically, based on the potential proximity between the wolves and the observer. This proximity facilitated identification and observation of individuals and retrieval of fecal samples. In general, the approximate location of wolf packs was determined initially using radio telemetry. Subsequently, a decision was made about which pack to continue viewing based on the potential proximity to the viewer, the ability to identify individuals and the feasibility of collecting fecal samples once the wolves moved away from the area where they were located. Once the focal pack was chosen and located, we watched from a vantage point using a 60x power spotting scope, moving positions as necessary throughout the day to keep the wolves in view. Because continuous observation was not always possible, we recorded the times that the wolves came into and out of view of the observer. Observations continued until the wolves went out of sight, darkness prohibited further viewing or collection of fecal samples took priority.

Individual wolves were identified by relative size, distinct coat and facial coloration or familiarity with distinct characteristics, such as a bent tail or barrel chest. In certain cases the presence or absence of a radio-collar facilitated identification. All individuals in the Druid pack were identifiable. In the Rose Creek and Leopold pack, approximately 1/3 of the individuals were identifiable (the proportion varied), always including the alpha pairs. Thus, for both sexes in all packs, we were able to distinguish

alpha from non-alpha (subordinate) individuals. The mean observation period was 1.91 hours, with the shortest being 0.25 hours and the longest lasting 8.67 hours.

Since individual identification was difficult for the Rose Creek and Leopold packs, behavioral sampling focused more heavily on the Druid pack. To establish rank order within the Druid pack and determine hourly rates of behaviors for individual pack members, we used all occurrences behavior sampling (Altmann 1974). We recorded initiator, recipient and winner/loser where appropriate for all aggressive, agonistic, affiliative and breeding behaviors (Table 1). The category "agonistic behavior" included any behavior related to dominance that did not involve physical contact. The category "aggressive behavior" included any dominance behavior that involved physical contact between wolves. For matings, we noted the initiator, recipient and duration of the mount. We defined the mating periods as the interval of time between the first and last day that we witnessed mating behaviors.

We used scan sampling in ten minute intervals to record presence or absence of each pack member to correct for biases in observability among individuals (Drickamer 1974). During scan samples we recorded the nearest neighbor for each individual and the distance between them to determine patterns of association between pack members. Using the scan sample data, we corrected the rates of behaviors to account for the proportion of time each individual was visible. We then calculated hourly rates of agonistic, aggressive, affiliative and breeding behaviors for individuals.

We determined dominance rank from aggressive and agonistic interactions in which a winner and loser could be determined. To obtain an ordinal scale of dominance,

we organized the data in a standard win-loss matrix (Martin & Bateson 1986), with a rank order that minimized the number of reversals of dominance interactions (when a subordinate wins an encounter with a normally dominant individual). This method yields an acceptable rank order for wolves, but it treats the hierarchy as a ladder with individuals on evenly-spaced rungs. That is, it does not estimate the *magnitude* of the difference in social status between wolves that are adjacent in a linear hierarchy. To obtain these relative distances, we used the Batchelder-Bershad-Simpson (BBS) scaling method (Jameson et al. 1999), a mathematical model of paired comparisons involving simple estimation procedures. This procedure yields probabilities associated with pairs of ranks, so that for any two animals, information on how likely one animal is to dominate the other is given as a probability. Using this method, an animal's scale position depends on 1) the proportions of wins and losses in its encounters with others and 2) the scale scores of the others it has met in agonistic encounters. An iterative procedure is used until scores converge (Jameson et al. 1999). Using this procedure, the difference in dominance between individuals can be quantified and tested for statistical significance. To calculate these, we utilized a java applet of the scaling algorithm described in Jameson et al. 1999 (<http://caspar.bgsu.edu/software/Java/1Hierarchy.html>). So that data could be pooled across both years, we transformed the dominance values obtained from the BBS method. We used a linear transformation that gave the highest-ranking individual a dominance value of 100 and maintained the relative distances between individuals in the hierarchy (see results, Table 5).

Fecal Sampling

During observations of the wolves, we recorded the locations of fecal samples and when possible, the identification of the wolf who defecated. Thorough descriptions of the location were recorded into a dictaphone and sketched in a field notebook and were later used to locate samples from known individuals once the wolves moved away. We sometimes used two-way radio communication between an observer and a retriever to recover the sample. The individual who witnessed the defecation would return to the observation point and with the use of the spotting scope, direct the retriever to the sample. The retriever also collected all other fecal samples encountered along the route. We used other opportunistic methods to collect fecal samples from unknown individuals of known packs. We found these samples by snow tracking the wolves and searching any areas where we had observed them, including the road and the vicinity of any wolf-killed carcasses when possible. When known, we recorded time of defecation, lag until collection and lag until freezing to test whether the elapsed time and degradation had a significant effect on cortisol concentration detectable in the samples. These lag times were tested as covariates in the statistical analysis of hormone concentrations. Samples were stored at -80°C until assay.

Extraction and Radioimmunoassay

We extracted steroids using slight modifications from published methods (Brown et al. 1994; Monfort et al. 1997; Wasser et al. 1994). Fecal samples were weighed, fully dried in a rotary evaporator, then re-weighed to determine water content. We boiled 0.2 g of dried feces in 10 ml ethanol for 20 min, and then centrifuged at 1500 rpm for 15 min. Centrifuge pellets were weighed to determine the amount of indigestible matter, primarily hair and bones, in each sample before discarding (the weights of these pellets resulted in an index of indigestible material in feces). Supernatants were decanted, dried under air in a warm water bath, rinsed with 2-3 ml ethanol, then vortexed for 15 sec, re-dried, and reconstituted in 1 ml absolute methanol. After the ethanol rinse, tubes were vortexed for 15 sec and placed in an ultrasonic glass cleaner for 15 sec to free particles adhering to tube walls. After reconstitution in methanol, the extract was vortexed for 1 min, placed in glass cleaner for 30 sec, then vortexed again for 15 sec. Fecal extracts in methanol were stored at -80°C . Of this extract, 25 μl was taken to assay.

We measured GCs with a coated-tube ^{125}I -cortisol assay from Diagnostic Products Corporation, which has little cross-reactivity to other steroids (maximum 0.2% for progesterone). We validated the radioimmunoassay for fecal cortisol using standard criteria (Cekan 1975). We checked for specificity by a test of parallelism; serial dilution of standards and fecal extracts yielded parallel changes in antibody binding for six points, to 10-fold dilution. We determined quantitative accuracy by measuring recovery of cortisol (25 μl at 5 - 500 ng/ml) added to fecal extracts. Recovery was $107\% \pm 2\%$ ($t = 55.9$, $P < 0.001$, $R^2 = 0.998$). Intra and inter-assay coefficients of variation (C.V.) for a

pooled control sample were 5.8% and 10.4% respectively. Sensitivity was 10 pg/tube. Each sample was assayed in duplicate yielding a within sample C.V. Some assays initially showed a high, within-sample C.V. If the C.V. was >15%, we repeated the assay up to four times in an attempt to reduce the C.V. When repeated measurements were made, the results were averaged and the resulting C.V. was based on all repeated measures. Hormone concentrations were expressed as ng cortisol/g dry feces. We calculated the percent water and the index of indigestible material in each fecal sample to test whether differences in diet affected the GC concentrations detectable in the samples.

Climate Data

We obtained weather data to test if snow pack and temperature conditions triggered a stress response in wolves. We obtained daily maximum and minimum air temperatures, snowfall and depth of snow on the ground from the Tower Falls climatological station in YNP (which is located in an area shared by the Rose and Druid packs). Snow water equivalent was calculated from these data (Farnes 1999) and utilized as an index of snowpack severity in the study area.

Statistical methods

We tested whether the composition of scat samples or the time lags between defecation, collection and freezing may have affected the cortisol concentration detectable in the samples. We included three procedural variables: percent water, indigestible index and the within sample C.V. as covariates in all further analyses of factors affecting GC levels (see results section). R^2 values reported are for multiple regressions including these procedural variables as covariates. Where other variables were controlled, they are noted. We used parametric statistical tests (ANOVA, multiple regression) after testing that assumptions were met. When data were log transformed this was noted. Means are reported with standard errors except where noted. T-tests are two-tailed except where noted. All statistical analyses were performed using STATISTICA, including tests of assumptions for parametric tests. To avoid pseudoreplication, we used a nested ANOVA with data from individually-known Yellowstone wolves that were resampled, which showed that fecal samples could be considered statistically independent units of analysis (Steel & Torrie 1980).

Table 1. Categories of Behavior Sampled During All-Occurrences Sampling.

Agonistic (Behaviors related to dominance that generally do not involve physical contact)

raised leg urination	muzzle bite
double scent mark	muzzle bite
ground scratch	pounce
tail position (up, level, down, tucked)	roll over
ears back	ride up
approach submissively	snarl
approach tail (up, level, down, tucked)	stand over
approach ears back	bare teeth
crouch	chin rest
stalk	chase
hackles up	inhibited bite
inguinal present	snap
lunge	growl

Aggressive (behaviors related to dominance that involve physical contact)

mob
body check
jaw spar
bite
pin
knock down
attack

Affiliative

Greet
group ceremony
rally
tail wag
approach with tail wag
muzzle lick
initiate play
play
play bow

Breeding

tail avert
mount
mount attempt
tie
thrust
sniff anal region
ride up
chin rest
lick

RESULTS

Procedural VariablesFecal collection, extraction and assay

We tested for potential effects on GCs of lag times between defecation and collection (DC) and between defecation and freezing (DF), percent water, proportion of indigestible material in feces, and the coefficient of variation (CV) for repeated within-sample measurements on cortisol concentration detectable in feces. The multiple regression was significant ($F_{5,274} = 5.45$, $p < 0.01$), but explained relatively little variance in GC levels- ($R^2 = 0.08$), which suggests that procedural variables were relatively unimportant, as would be hoped. The effect of lag time was not significant for our samples (DC: partial $b = -0.19 \pm 0.33$, $t_{(274)} = -1.61$, $p = 0.11$; DF: partial $b = 0.15 \pm 0.01$, $t_{(274)} = 1.49$, $p = 0.14$). Since we worked primarily in winter, a majority of fecal samples remained frozen in snow prior to collection. After collection, samples were placed in a conventional freezer within six hours with few exceptions. Typically within two weeks, the samples were then transferred to a -70°C freezer and stored there until extraction. The proportion of indigestible material (partial $b = -0.73 \pm 0.18$, $t_{(274)} = -4.01$, $p = 0.00$) and CV (partial $b = 0.02 \pm 0.01$, $t_{(274)} = 2.01$, $p = 0.04$) were significant, whereas percent water (partial $b = 0.74 \pm 0.44$, $t_{(274)} = 1.70$, $p = 0.09$) was not. We included all three of these procedural covariates in further analyses of factors affecting GC levels. While percent water was not significant in this regression, in subsequent analyses the inclusion or exclusion of percent water affected the correlation of other variables with GC levels.

Behavioral Observations and Sample Collection

Table 2 shows a summary of samples collected and hours of observation for the three packs studied. A total of 351 fecal samples and 377 hours of behavioral observations were used in analyses. Of these 351 samples, 117 came from known individuals from these packs or lone animals within the study area. The remaining 227 samples came from the same three packs, but the individual was either not recognizable or the defecation was not directly observed. These 'unknown' samples did not provide information on rank effects, but did let us examine the effects of pack size, environmental effects, variation among years, and composition of the dropping. In all three packs, for both years of the study, we could identify the alpha pair and distinguish them from the subordinates. For the Leopold and Rose Creek pack we could identify approximately half of the individuals in the pack. For the Druid pack, we were able to identify all individuals and thus determine their specific rank order. Since the Druid pack was also the most visible of the three packs, we were able to collect more detailed behavioral data (271h) than with the Leopold (32h) and Rose Creek (74h) packs. For both years of the study, the only adult male in the Druid pack was the alpha male (021M). In both years, his male offspring, upon reaching approximately 16 months of age, dispersed from the pack. There were five adult females in the Druid pack for both years of the study until May 2000, when the alpha female (040F) was killed by other wolves.

Dominance Determinations

Win-Loss Matrix

Rank order matrices for Druid pack in 1999 and 2000 are presented in Tables 3 and 4, respectively. Though there is a separate dominance hierarchy for males and females in wolves, we included both sexes in these rank orders to provide more information on the dyadic encounters involving the females in the pack. For example, the interactions of two females with a male might reveal something about the rank of the two females, relative to one another. For both years, the only males in the pack were the alpha male (021M) and one of his pups (163M in 1999 and an uncollared pup in 2000) and it was evident that they were subordinate to 021M. The rank order of the females was identical with or without the inclusion of the males in the matrix. The rank order was consistent between years for the females, with 040F as the alpha and 042F as the beta, followed by 105F, 103F and then 106F as the lowest ranking. The proportion of combined aggressive and agonistic encounters each wolf was involved in did not show a strong relationship to rank order in either 1999 or 2000 (Fig. 1). Though the dominant animals won more often than subordinates, they did not fight more often than subordinates. In 1999, the alpha female, 040F had the highest number of agonistic encounters, while in 2000, the lowest ranking female, 106F was involved more often than any other wolf in the pack.

Table 2. Pack summary of behavioral observations and fecal samples of wolves collected from three packs between January 1999 and July 2000 in Yellowstone National Park. The column 'other' represents samples collected from lone individuals who were not in the three packs studied but were within the study area.

Data Types	Wolf Packs			Other	Total
	Druid	Leopold	Rose Creek		
Fecal sampling					
Number of fecal Samples from known individuals	89	10	18	7	124
Number of fecal samples from unknown individuals	138	24	65	0	227
Total fecal samples	227	34	83	7	351
Behavioral observations					
Maximum number observed	8	12	16		36
Maximum number Identifiable	8	5	7		20
Number of collared wolves: January 1999	7	5	9		21
Number of collared wolves: January 2000	5	6	6		17
Number of Observation periods	125	11	35		171
Total hours Observed	271	32	74		377
Mean observation Period (hrs)	2.2	2.9	2.1		1.91

Table 3. Druid pack 1999 rank order win-loss matrix

1999 Loser	Winner							Total Loss
	021M	040F	042F	105F	103F	106F	163M	
021M	0	1	0	0	0	0	0	1
040F	3	0	0	0	0	0	0	3
042F	2	11	0	0	0	0	0	13
105F	2	1	1	0	0	0	0	4
103F	5	5	1	4	0	0	2	17
106F	2	2	1	2	1	0	0	8
163M	3	1	2	3	1	0	0	10
Total Wins	17	21	5	8	2	0	2	

Table 4. Druid pack 2000 rank order win-loss matrix.

2000 Loser	Winner							Total Loss
	040F	021M	042F	105F	103F	106F	PUP	
040F	0	2	0	0	0	0	0	3
021M	3	0	1	0	0	0	2	5
042F	26	2	0	0	0	0	1	29
105F	16	6	1	0	0	0	1	24
103F	8	5	14	14	0	0	2	41
106F	12	12	12	17	52	0	3	108
PUP	4	13	4	1	1	1	0	24
Total Wins	69	40	32	32	53	1	7	

BBS Method

The scale scores using the BBS method yielded a similar rank order to the win-loss matrix method (Table 5). The alpha male (021M) and female (040F) had the highest scores for both years and the order of the lower ranked pack members was consistent except that 105F had the second highest female rank for 1999. After watching the pack dynamics for two years, we were confident that the beta female in the pack was 042F, the mother of 105F, so we used the ordinal rank order resulting from the matrix in further behavioral analyses. (As is typical for a beta wolf, 042F took over the alpha position once 040F died in May, 2000.) We also used BBS to rank the females independently, excluding the males. The BBS rank order is consistent and the relative distances between the females are similar to the order when the males are included (Table 5). The method is not meaningful unless three or more individuals are ranked, so we could not run the model with just the males in either year. The transformed scores from the BBS method were used to analyze relationships between rank and GC levels using rank as a continuous variable.

Figure 1. Proportion of dominance (both aggressive and agonistic combined) encounters that each wolf in Druid pack was involved in for 1999 and 2000. The wolves are ordered by rank from left to right on the x-axis. The first two wolves 021M and 040F are the male and female alphas and the wolf on the far right (163 or PUP) is the only subordinate male in 1999 and 2000 respectively.

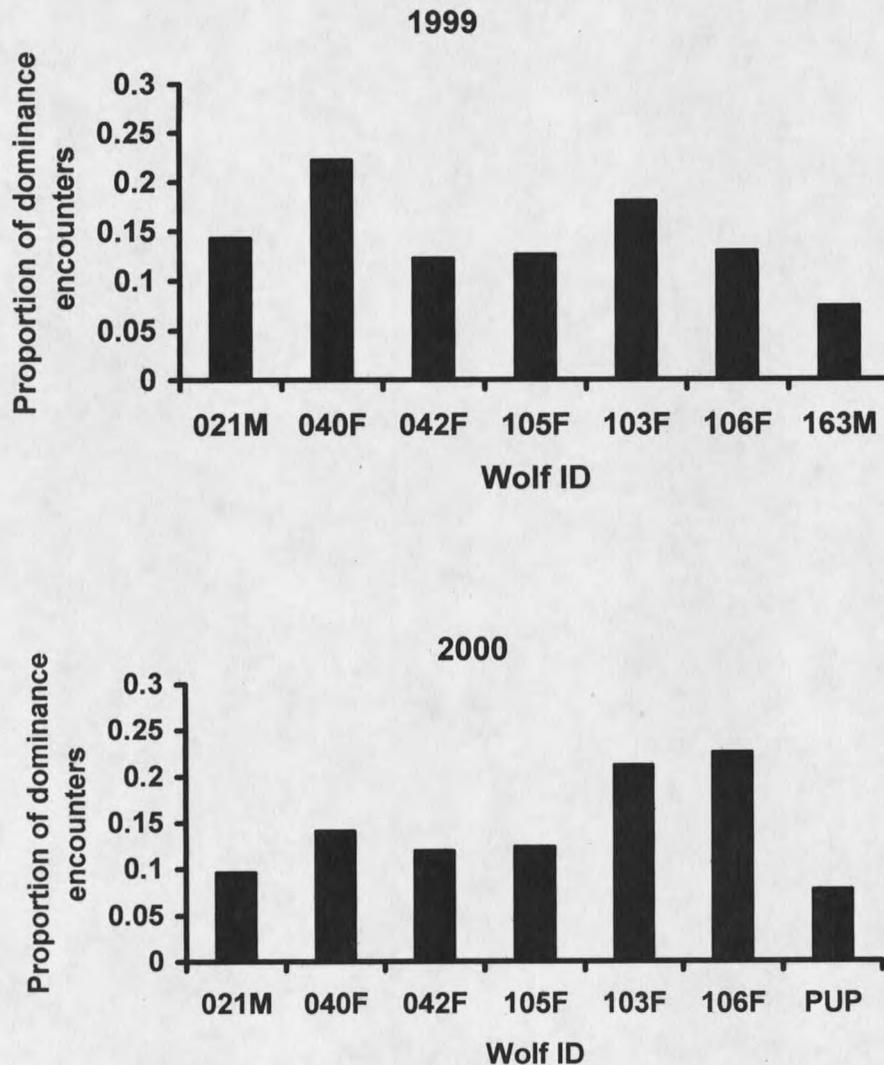


Table 5 Batchelder-Bershad-Simpson (BBS) scaling method scores for Druid Pack in 1999 and 2000. Both raw scores and transformed scores are presented. Scores are shown for the full pack in the left column and for just females in the right column. When females are treated separately, their order is maintained and the relative distances between scores are consistent with scores for the pack.

1999 BBS method

IDs	Raw		Transformed	
	Pack	Females	Pack	Females
021M	1.330	-----	100	-----
040F	1.171	1.364	95.867	100
105F	0.1692	0.125	69.826	69.817
042F	-0.299	-0.269	57.655	60.219
103F	-1.171	-1.154	34.988	38.660
163M	-1.204	-----	34.130	-----
106F	-1.517	-1.741	25.994	24.361

2000 BBS method

IDs	Raw		Transformed	
	Pack	Females	Pack	Females
040F	1.713	1.980	100	100
021M	1.365	-----	92.134	-----
042F	0.460	0.618	71.668	71.027
105F	0.308	0.563	68.241	69.858
PUP	-0.331	-----	53.791	-----
103F	-0.362	-0.285	53.101	51.819
106F	-1.711	-1.721	22.604	21.272

Rank and Glucocorticoid levels

To examine the effects of rank, we controlled for variation among packs and years. Cortisol concentration values were log transformed prior to analysis to obtain normality.

Rank as a Categorical Variable

When rank is treated categorically as either alpha or subordinate, social status had a significant effect on GCs (Fig. 2: $F_{1,95} = 4.061$, $p = 0.047$), with alpha animals having higher mean levels (1876 ± 286.7 ng cortisol/g dry feces) than subordinates (1413 ± 222.1 ng cortisol/g dry feces). This pattern was consistent between years (interaction: $F_{1,95} = 1.31$, $p = 0.25$) across packs (interaction: $F_{2,95} = 0.68$, $p = 0.51$) and for both sexes ($F_{1,83} = 0.276$, $p = 0.60$) (Fig.3). Initially we did not include the beta with the subordinates since other studies (e.g. Creel et al.1997) suggest that the endocrine profiles of betas are sometimes different than other subordinates. When we included the beta female from the Druid pack in the comparison of GC levels between ranks, we found an interesting pattern. The beta female's mean cortisol concentration (3466 ± 286.7) was higher than both the alpha and subordinate levels. The sample size ($n = 9$) for betas is small and the variance is large. Nonetheless, it is interesting to note that though the beta animal is behaviorally similar to other subordinates, her mean GC levels are more similar to the alpha's levels (Fig.4).

Rank as a continuous variable

We also used the BBS scores to analyze the relationship between GCs and rank, treating rank as a continuous variable. So that data from both years could be pooled, we used a linear transformation of the BBS values that gave the highest-ranking individuals a dominance value of 100 and the relative distance between individual scores was maintained. When rank is treated in this manner, it explains little variation in GC levels (Fig. 5: $R^2 = 0.09$, partial $b = 0.004 \pm 0.005$, $t_{(77)} = 0.792$, $p = 0.431$). When rank is treated as a categorical variable we detect a difference between alphas and non-alphas whereas treating rank as a continuous variable reveals no pattern. Recall that the BBS method uses proportions of wins and losses in the dominance calculations to determine an animal's scale position. It is notable that GCs are associated with social status when rank is treated as a simple dichotomy, but not when rank is measured as a continuous variable that depends directly on proportions of wins and losses or rates of winning and losing (GCs are not related to rates of aggression, winning or losing: see below). These patterns suggest that the critical factor affecting GC levels is simply whether or not a wolf is at the top of the hierarchy, rather than the behavioral details involved in maintaining this position.

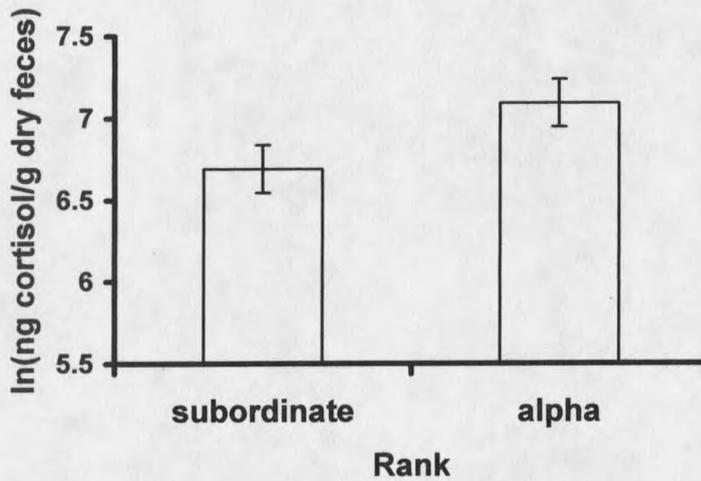


Figure 2. Cortisol concentration level comparison between subordinates and alphas pooled across the Druid, Leopold and Rose Creek packs for both years. Social status has a significant effect on cortisol concentration ($F_{(1,95)} = 4.06$, $p = 0.047$), with dominant wolves having higher mean levels (1876 ± 286.7 ng cortisol/g dry feces) than subordinates (1413 ± 221.0 ng cortisol/g dry feces).

