

## The relationship between social stress and dominance is seasonal in greylag geese

KURT KOTRSCHAL\*, KATHARINA HIRSCHENHAUSER\* & ERICH MÖSTL†

\*Konrad-Lorenz-Forschungsstelle and Institute for Zoology, Ethology Department,  
The University of Vienna

†Institute for Biochemistry, University of Veterinary Medicine, Vienna

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**Abstract.** Studies of captive animals have suggested that social stress affects subdominants, whereas recent data from the wild have revealed that stress mainly affects dominants. We used a non-invasive approach to investigate, for the first time in a social bird, the circannual stress–dominance relationships between low-ranking single males, intermediately positioned paired ganders without offspring and high-ranking paired males with offspring from a flock of semi-tame, free-ranging greylag geese, *Anser anser*. We collected 933 faecal samples from 43 individuals, 12 singletons, 18 paired males without offspring and 13 paired males with offspring over an entire year and analysed them for corticosterone metabolites by enzyme immunoassay. During the mating season (February–April), singletons had marginally higher corticosterone than paired males ( $P < 0.1$ ), whereas during the parental season (May–January), the paired males with offspring had significantly higher corticosterone than both paired males without offspring and singletons. All three male categories had significantly higher corticosterone during the mating season than during the rest of the year. These results suggest that social stress in ganders is caused mainly by competition between males and by constrained access to females during the mating season, but by parental commitment during the rest of the year. We suggest that dominance per se may not be a direct cause of stress. Rather, the amount of social stress may co-vary with the behavioural investment individuals need to make to optimize their fitness and with the relationship between such demands and the individuals' rank positions. This relationship seems to be seasonal in geese.

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Studies, mostly of captive animals, have generally revealed an inverse relationship between dominance and stress (captive rabbits, *Oryctolagus cuniculus*: Girolami et al. 1996; captive tree shrews, *Tupaia belangeri*: von Holst 1969; captive timber wolves, *Canis lupus*: McLeod et al. 1996; free-living white-crowned sparrows, *Zonotrichia leucophrys*: Wingfield & Farner 1978; Rohwer & Wingfield 1981). Techniques to analyse steroid hormones from faeces or urine (Bercovitz et al. 1982; Möstl et al. 1987) allow the relationships between social status and stress in unrestrained animals to be investigated. As an additional benefit, non-invasive sampling renders handling of

the animals unnecessary and stress measurements are therefore unaffected by the investigator. Field data on dwarf mongooses, *Helogale parvula*, and African wild dogs, *Lycaon pictus*, obtained with this new methodology contradict the conventional wisdom of an inverse relationship between dominance and stress and show that social stress may fall more heavily on dominants (Creel et al. 1996).

A flock of greylag geese, *Anser anser*, was established in the valley of the river Alm (Austria) by Konrad Lorenz in 1973. All individuals have been marked with coloured leg rings and have been regularly monitored for social interactions ever since. By analysing corticosterone equivalents from faeces of this semi-tame, free-ranging flock of geese, we aim to contribute another data set to the current debate on the social causes of stress. These social birds exhibit distinctly seasonal patterns of reproduction and flocking (Rutschke

Correspondence: K. Kotrschal, Konrad-Lorenz-Forschungsstelle für Ethologie, A-4645 Grünau 11, Austria (email: klf.gruenau@telcom.at). E. Möstl is at the Institut für Biochemie, Veterinärmedizinische Universität, J.-Baumann-Gasse 1, A-1210 Wien, Austria.

1982). Therefore, the circannual relationships between dominance and stress may not remain constant. During the spring mating season, for example, stress in paired males may depend on challenges by rivals (Wingfield et al. 1990), but stress levels in the majority of such males are probably still lower than in the single males (singletons), which generally have little access to females. After the young hatch this pattern may reverse. Then, relatively high levels of vigilance and agonistic interactions with other flock members shown by paired males with offspring (Lamprecht 1986; Kotschal et al. 1993) may cause higher stress levels in these males than in singletons. We therefore specifically ask how seasonal social changes may affect stress in males of different social status and dominance. We focus on paired males with offspring and on singletons (Fig. 1) and use paired males without offspring for comparison.

## METHODS

The Grünau flock of 140 greylag geese spends the day at meadows close to the research station and uses a lake, 10 km to the south, as a night roost. As the birds are habituated to the presence of observers within the flock, we could collect individual droppings.

We sampled faeces of 12 singletons, 13 paired males with offspring and 18 paired males without offspring (total: 43 individuals) over a complete annual cycle, when possible. The present results are based on the analysis of 933 individual faecal samples collected in 1993–1994, 276 from singletons, 393 from paired males with offspring and 264 from paired males without offspring.

Rank within a flock of geese depends on social bonding (Lamprecht 1986; Black & Owen 1987). We chose the categories family males (i.e. paired males with offspring) and singletons (i.e. males with no social bond as defined by the lack of a companion with which a triumph-ceremony is performed; Lorenz 1988) for comparison because the former are highest, and the latter lowest, in rank among all males. Paired ganders in the company of their mate but without offspring are subordinate to family males, but dominant over singletons. Gander categories were therefore defined by bonding and rank. Age is generally unrelated to bonding or rank; however, single-

tons, the majority of them widowers, were significantly older than family ganders or paired ganders without offspring. Single males were 6–26 years old ( $\bar{X} \pm \text{SE} = 16.5 \pm 5.5$ ), family males 3–23 years old ( $9.0 \pm 5.8$ ) and paired ganders without offspring 3–11 years old ( $6.5 \pm 2.5$ ).

On average we collected 20 faecal samples per individual over the entire year, which was divided into 13 phases (for definitions see Fig. 1) and two seasons: 'mating' (phase 1, courtship, to phase 6, second half of incubation) and 'parental' (phase 7, hatching, to phase 13, pre-courtship; even though the term 'parental' applies only to the family males). We sampled varying numbers of individuals from the total of 46 per phase and season (see Results). We compared individual mean corticosterone values per phase using two-tailed tests: Mann-Whitney *U*-test for within-season, between-category comparisons and Wilcoxon for between-season, within-category comparisons.

Gut passage time in geese is as fast as 2–3 h and individuals may defecate more than once per h. Steroid metabolites are excreted via hepatic or renal pathways. Because it is not possible to separate faeces and urine in goose droppings, we analysed both together. As in other field studies (Creel et al. 1996), blood was not sampled and thus corticosterone plasma levels are unknown. A parallel excretion study with domestic geese, which received systemic injections of  $^3\text{H}$  corticosterone (E. Möstl, unpublished data), however, revealed that metabolite excretion started a few minutes after the injection and peaked 1–2 h later. In agreement with studies by Cockrem & Rounce (1994; plasma-faeces relationships in domestic fowl, *Gallus gallus domesticus*) and by Cook et al. (1996; serum-saliva relationships in swine, *Sus scrofa domestica*), another series of experiments with domestic geese (K. Kotschal & P. Peczely, unpublished data) showed deterministic relationships between plasma steroids and metabolites in the faeces. Therefore, we are confident that the faeces contained an integrated, proportional record of the plasma corticosterone levels approximately 1 h before defaecation. In addition, the patterns found (see Results) are biologically plausible.

We assayed faecal corticosterone equivalents by enzyme immunoassay (Möstl et al. 1987) using an antibody against corticosterone-3-CMO: BSA (working dilution 1:40 000). As a label corticosterone-3-CMO-dioxaoctane-biotin (Palme

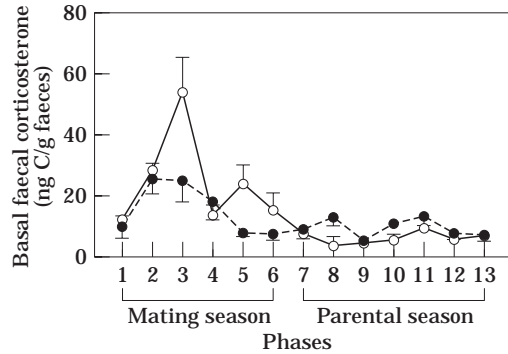
& Möstl 1993) was used (working dilution 1:200 000). The sensitivity of the assay was  $<2$  pg. The assay showed cross-reactions with 4-pregnene-17 $\beta$ , 17 $\alpha$ , 21-triol-3, 20-dione (5.0%), with 5 $\alpha$ -pregnane, 3 $\alpha$ , 11 $\beta$ , 17 $\alpha$ , 21-terol-20-one (0.15%) and with 5 $\beta$ -pregnane, 3 $\alpha$ , 11 $\beta$ , 21-triol-20-one (25%). Faecal samples (0.5 g) were extracted with 1 ml water plus 1.5 ml methanol by vortexing (30 min). Afterwards, an aliquot of each sample was evaporated to dryness and re-dissolved in acetate buffer (pH 4.8). Low recovery of active antigen after ether extraction suggested that more than 95% of the corticosterone metabolites were present in a conjugated form. HPLC revealed that enzymatic hydrolysis with a mixture of  $\beta$ -glucuronidase/arylsulphatase (Merck 4114) increased the recovery to 23%, most of which was detected by the antibody. We assayed samples only once. We determined intra- and inter-assay variations by using homogenized pool samples. The mean intra-assay coefficient of variation was 13.9%, and the mean inter-assay coefficient of variation was 14%.

## RESULTS

Over 1 year corticosterone metabolite concentrations detected by the antibody in faeces of the family ganders varied between 0.4 and 81.2 ng/g faeces. In the singletons variation was between 0.6 and 71.4 ng/g.

### Within-phase Comparison, Singletons versus Family Males

A plot of faecal corticosterone patterns over the 13 phases of the year (Fig. 1) suggests that during the mating season, singletons generally had higher levels of corticosterone than family ganders and vice versa during the parental season. A pair-wise, within-phase comparison of corticosterone levels (sample means/individual/phase, for  $N$  see legend to Fig. 1) of high-ranking family males versus low-ranking singletons revealed only one significant difference, in phase 5 (first 2 weeks of incubation, Fig. 1), where singletons had significantly higher levels than family males (Mann-Whitney  $U$ -test:  $Z = -2.01$ ,  $N_1 = 6$ ,  $N_2 = 5$ ,  $P < 0.05$ ). Clumping together of similar phases (i.e. phase 2 with 3 or phase 5 with 6) did not change these results, but decreased the temporal resolution and was there-

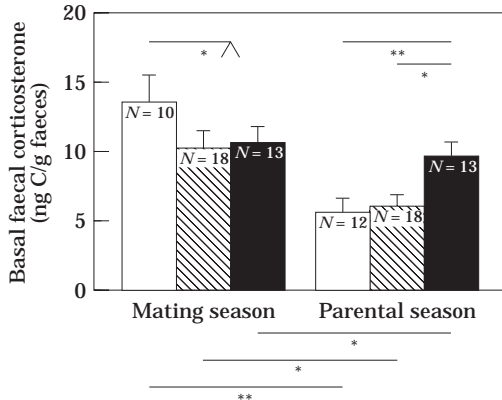


**Figure 1.** Annual corticosterone (C) levels as determined by enzyme immunoassays from the faeces of male greylag geese. ○: Low-ranking singletons; ●: high-ranking family ganders (i.e. paired males with offspring). Mean values  $\pm$  SE of ng C/g faeces are plotted for 13 phases in the greylag's annual cycle. A total of 12 singletons and 13 family males were sampled throughout the year. Number of individuals sampled per phase is as follows. 1: Courtship period in February (9 subdominant singletons, 13 dominant family males); 2: fourth to third week before laying (6/11); 3: the 2 weeks preceding the first egg (5/7); 4: egg laying, duration approximately 2 weeks (6/2); 5: first 2 weeks of incubation (5/6); 6: third and fourth week of incubation (6/4); 7: hatching (end of April to beginning of May) and the first 2 weeks thereafter (5/8); 8: moult in June/July (5/9); 9: summer flock, August and September (12/13); 10: pre-migratory phase, October (6/12); 11: 'migratory restlessness', end of October to mid November (6/6); 12: winter flock, end of November and December (7/10); 13: pre-courtship-phase, January (6/12).

fore avoided. An analysis of intra-individual corticosterone variability within season (Wilcoxon) revealed no significant result. Therefore, the results were not due to only a few birds with high corticosterone levels.

### Within-season Comparison, Singletons versus Family Males

When analysed within season (Fig. 1: mating, phases 1–6; parental, phases 7–13), family males had significantly higher levels of corticosterone than singletons during the parental season (Mann-Whitney  $U$ -test:  $Z = -2.83$ ,  $N_1 = 13$ ,  $N_2 = 12$ ,  $P < 0.01$ , Bonferroni corrected; Fig. 2). However, the apparently higher corticosterone values of the singletons during the mating season remained non-significant compared with the



**Figure 2.** Mean corticosterone (C) values  $\pm$  SE of single males, paired males without offspring and family males (i.e. paired males with offspring) during both mating and parental seasons.  $\square$ : Singletons;  $\square$  (hatched): paired males;  $\blacksquare$ : family males. The number of individuals per group is shown. Significant differences between groups are indicated by lines: \* $P < 0.05$ , \*\* $P < 0.01$  all two-tailed. Mann-Whitney  $U$ -test (Bonferroni corrected) for comparisons between categories within season (lines above bars), Wilcoxon for comparisons within category, between seasons (lines below bars).

family males (Mann-Whitney  $U$ -test:  $Z = -1.05$ ,  $N_1 = 10$ ,  $N_2 = 13$ , NS).

#### Within-category, Between-season Comparison, Mating versus Parental

A comparison within male categories between seasons (Fig. 2) revealed that all male categories, singletons, paired males without offspring and family males, had significantly higher corticosterone levels during the mating season than outside (Wilcoxon: singletons:  $Z = -2.7$ ,  $N = 10$ ,  $P < 0.01$ ; paired males with offspring:  $Z = -2.43$ ,  $N = 13$ ,  $P < 0.02$ ; paired males without offspring:  $Z = -2.42$ ,  $N = 18$ ,  $P < 0.02$ ).

#### Paired Males versus Singletons and Family Males

A comparison of single and family males with an intermediate social category of males, paired ganders without offspring, is revealing with respect to the possible social causes of the stress patterns found (see Discussion). Within the mating season, corticosterone levels of these ganders were close to those of the family ganders, whereas during the parental season they were

close to those of singletons (Fig. 2). During the parental season both single males and paired males without offspring had significantly lower corticosterone levels than family males (Mann-Whitney  $U$ -test:  $Z = -2.83$ ,  $N_1 = 12$ ,  $N_2 = 13$ ,  $P < 0.01$  and  $Z = -2.46$ ,  $N_1 = 18$ ,  $N_2 = 13$ ,  $P < 0.05$ , Bonferroni corrected). Neither the family ganders nor the paired ganders without offspring alone had significantly different levels from the singletons during the mating season (Mann-Whitney  $U$ -test:  $Z = -1.05$ ,  $N_1 = 13$ ,  $N_2 = 10$ , NS and  $Z = -1.7$ ,  $N_1 = 18$ ,  $N_2 = 10$ ,  $P < 0.1$ ). However, when the family males were grouped together with the paired males without offspring (which is feasible, because offspring of the previous year split from their parents at the onset of the reproductive season, allowing the family males to be considered as previously successful, paired males), they had lower corticosterone levels than the 'unpaired' singletons (Fig. 2; Mann-Whitney  $U$ -test:  $Z = -1.93$ ,  $N_1 = 31$ ,  $N_2 = 10$ ,  $P = 0.05$ ).

## DISCUSSION

Our results show that the relationship between dominance and stress in greylag ganders is not a simple one. Individual stress load is not an attribute of rank per se, but dominance seems to modulate stress in accordance with seasonal changes in investment in reproduction, which again depends on social status. The significantly higher corticosterone levels of all gander categories (singletons, paired without offspring and family males), during the reproductive season (Figs 1 and 2), for example, may reflect competition for females, high sexual activity and frequent agonistic interactions. However, unpaired males still had higher corticosterone levels than both categories of paired ganders, which may be caused by a conflict between their sexual motivation and their limited access to females because of their low rank. Attempts by singletons to win a mate are usually unsuccessful and they are frequent targets of attacks (unpublished data).

After the mating season, singletons emphasize feeding and survival without being constrained by social bonds. High-ranking family ganders, however, stay close to their mates and offspring all year and are significantly more vigilant and aggressive than singletons (Lamprecht 1986; Black & Owen 1987; Kotschal et al. 1993;

Waldenberger & Kotrschal 1993). Therefore, the significantly higher stress hormone levels of the dominant family males throughout the parental season than both singletons and paired males without offspring (Figs 1 and 2) probably result from paternal commitment, not from permanent mate guarding, because stress levels of the paired ganders without offspring were close to those of the singletons during the parental season. In short, the circannual patterns of variation of social stress in ganders seem mainly to be caused not by limited access to females during the mating season, but by paternal effort during the rest of the year.

Accordingly, high stress levels of subdominants in captive populations may not be caused directly by low rank, but may rather be due to their inescapable exposure to dominant individuals, especially as field studies have shown that stress levels tend to be higher in dominants (Creel et al. 1996). Therefore, dominance may be considered a co-variable rather than a direct causal factor of stress. The 'costs of dominance' (Silverin 1986; Beletsky et al. 1992; Altman et al. 1995; Frank et al. 1995; Packer et al. 1995; Wasser 1995) and subdominance may be regarded as costs of behaviour and conflict, modulated by rank. This interpretation complements conclusions reached by von Holst (1969 and unpublished data, captive tupaia, *Tupaia belangeri*) and Sapolsky (1992, wild baboons, *Papio anubis*) that it is not the dominance position itself, but the way it is achieved and maintained that determines physiological costs. Also, we agree with the earlier insight that 'the adrenal response to stress is . . . not a simple reflex action but is modified according to experience' (Harvey et al. 1984).

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