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Endocrine and Behavioural Responses of Male Greylag Geese (*Anser anser*) to Pairbond Challenges during the Reproductive Season

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Abstract

Although greylag geese *Anser anser* establish long-term monogamous pairbonds, some of the existing pairs do split up (divorce) and new pairs are formed during the annual spring mating period. In this study, male greylag geese which were involved in the challenge of an existing pairbond (challenged males and challengers) were regarded as 'natural experimental' groups and compared with males in stable pairbonds (unchallenged males and male-paired males, a common male strategy when the availability of females is low). In total, 37 males were investigated. The analysis included a description of the seasonal patterns of hormone levels, aggression and courtship. We tested whether hormone levels correlated with aggressive and courtship behaviours. Finally, we compared hormonal and behavioural patterns amongst the four groups. Immunoreactive testosterone (T) and corticosterone (B) equivalents were measured in faecal samples. Individual hormone levels were correlated with frequencies of agonistic male–male interactions and with frequencies of male–female courtship. During early mating and pre-laying phases, T was at its seasonal maximum, which may have masked hormone–behaviour correlations. During egg-laying, at the onset of seasonally decreased T, agonistic male–male interactions and the frequencies of courtship behaviour were significantly correlated with T. Unchallenged males had higher rates of agonistic interactions than any other males. However, unchallenged and challenged males tended to excrete T at higher levels than challengers. The high rates of being attacked and elevated levels of faecal B were indicative of the social conflict experienced by challengers. No hormonal differences were observed between heterosexually paired males and male-paired males. In summary, pairbond status and situations of social conflict had a modulating effect on T and B; however, in this study, the two hormones seem to be affected independently of one another.

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Introduction

Androgens have been shown to be involved in courtship and sexually motivated aggressive behaviour in male birds (Balthazart & Hendrick 1976; Pröve 1978; Farner & Wingfield 1980; Balthazart 1983; Bluhm et al. 1984; Bluhm 1988). Conversely, androgen levels may, in a 'positive' feedback loop, be stimulated by social interactions, particularly in successful males (Lehrman et al. 1961; Harding & Follett 1979; O'Connell et al. 1981; Hutchison & Hutchison 1983; Akesson & Raveling 1984; Wingfield & Wada 1989). Aggression and testosterone (T) are, however, not always correlated (Pröve 1978; Tsutsui & Ishii 1981; Sapolsky 1983; Dittami & Reyer 1984; Wingfield & Ramenofsky 1985). T is directly involved in aggression associated with reproduction, such as in mate guarding behaviour. The formation of social relationships was found to reduce the degree to which aggression was linked with T in the Japanese quail *Coturnix japonica* (Ramenofsky 1984). In free-living male birds, the behavioural effect of high circulating levels of T was strongest in situations of social instability, such as during pair formation or during challenges by a conspecific male (Wingfield 1990; Wingfield et al. 1990; Dittami et al. 1999). Thus, situations of social conflict may have a modulating effect on circulating levels of T (Oliveira et al. 1996).

Individual T and corticosterone (B) secretion (Schwabl 1995; Wingfield et al. 1997; Yehuda 1997) can be stimulated independently (Silverin 1998). B plays a role in the mobilization of energy reserves (Akesson & Raveling 1981; Silverin 1990), as well as in social interactions (Farner & Wingfield 1980; Wingfield & Silverin 1986; Frigerio et al. 1997). Social status, for example, modulates the pituitary–adrenocortical stress response (Creel et al. 1993, 1997; Altmann et al. 1995; Knapp & Moore 1996; Kotschal et al. 1998; Silverin 1998).

During the mating season in Feb. and Mar., T levels of greylag geese *Anser anser* are at their annual maximum (Akesson & Raveling 1984; Hirschenhauser et al. 1997, 1999a,b), in correspondence with frequencies of social interactions and numbers of pair formations (Dittami 1981; Lamprecht 1986; Owen et al. 1988). In this study, we analysed faecal hormones and recorded behaviour of males that were competing for pairbond females (challenged males and challengers). The aim was to investigate individual differences in T, B and social behaviour throughout different phases of the reproductive season. Greylag geese live in long-term monogamous pairs (Black et al. 1996). Pairbond formation in geese is expressed through courtship displays, aggressive conflicts and triumph ceremonies (Collias & Jahn 1959; Bluhm et al. 1984; Lamprecht 1986; Black & Owen 1988; Owen et al. 1988; Choudhury & Black 1993). In the Grünau flock of nonmigratory, free-living greylag geese, the reproductive season of 1995 was a socially dynamic period. Challenged (paired) males and unpaired challengers competing for bonded females were

regarded as the 'experimental' groups to be compared with males in stable pair-bonds, unchallenged males and male-paired males (Huber & Martys 1993).

We expected seasonal changes in all parameters over the sampling period (early mating, pre-laying, laying, incubation and hatching). On top of seasonal T fluctuations (Dittami 1981; Akesson & Raveling 1984; Hirschenhauser et al. 1997, 1999a,b) and in accordance with the challenge hypothesis (Wingfield et al. 1990), androgen responsiveness should be variable during phases of seasonally decreased T, but not during mating phases when T is at its physiological maximum. The question was, whether agonistic male–male interactions were modulating individual T and B, and whether these were correlated with frequencies of male–female courtship interactions (Wingfield 1990; Wingfield et al. 1990). Challengers and challenged males were frequently involved in competitive agonistic and courtship interactions and were therefore expected to have higher T levels than the unchallenged males. Furthermore, we tested whether the rates of being attacked by conspecifics were reflected in the levels of faecal B in challenged males and challengers. Therefore, correlations between hormones and behaviour were compared among social categories for the entire sampling period, as well as between different reproductive phases.

Methods

Animals

The free-living flock of greylag geese studied was introduced into the valley of the river Alm in Grünau, Upper Austria (Lorenz 1979). From Aug. to Feb., the geese can be found on meadows and ponds close to the research station. The flock spends the night on a lake 8 km to the south. To standardize spatio-temporal patterns, supplementary pellet food is provided twice a day throughout the year. In 1995, flock size was 124, including 36 pairs. All birds are individually marked with coloured leg bands. Because the geese are habituated to the close presence of humans, individual behavioural data and faecal samples can be collected year-round and without disturbance to the flock.

In 1995, the faeces and behaviour of 37 males were monitored throughout the reproductive season (Feb. to end of Apr.), i.e. during mating, egg-laying, incubation and hatching. Males were chosen depending on their involvement in challenge situations for access to females and/or when involved in the formation of a new pair. We divided males into four social categories: paired males who were challenged by a competitor for access to their female we referred to as 'challenged males' (n = 6). Unpaired males challenging a paired male and attempting to mate-guard the pairbond female were termed the 'challengers' (n = 6). Paired males which were not challenged by a competitor ('unchallenged males': n = 18) were considered as 'control' males. For further comparison with heterosexually paired males we classified ganders from male–male pairs ('male-paired males': n = 7).

Behaviour Sampling

Social interactions (courtship, pair formations and fights) among flock members are continuously monitored year-round. Individual behaviour was recorded

by 10-min continuous focal sampling around the morning and afternoon feeding times, when geese were at their peak of diurnal activity. A total of 460 protocols were collected. On average, 12 protocols per individual were analysed. We examined male–male interactions and male–female interactions. Agonistic male–male interactions were divided according to the focal male's initiative role (1), including a number of behaviours such as threatening, attacking, fighting and flight chasing a conspecific or when the focal male was the recipient of an attack (2). Agonistic units were only recorded when the target individual showed a behavioural response: retreat or fight back (Raveling 1970).

The recorded male–female interactions may be considered as two different phases of courtship during the pair formation process. The Angled Neck display (3) can be observed during the first phase of pair formation (Black & Owen 1988). Angled Neck display was recorded when a male pointed its beak towards the ground while walking in a conspicuously synchronous way behind, or in parallel to, a female (Fischer 1967; Prevett & MacInnes 1980). In contrast to the Triumph Ceremony, the Angled Neck display is only shown during pair formation (Lamprecht 1987; Black & Owen 1988; Mausz et al. 1992). The Triumph Ceremony (4) is a behavioural sequence initiated by the male attacking a nearby conspecific and returning to his partner with wing-flapping and cackling (Fischer 1965; Raveling 1970). Therefore, a positive correlation between aggressiveness and Triumph Ceremony may be expected. However, not only the winner of an agonistic encounter, but also the loser may perform a Triumph Ceremony (Radesäter 1974). It is interpreted as instrumental in strengthening or maintaining a pairbond (Fischer 1965; Lorenz 1974; Radesäter 1974), but may also serve to advertise an existing pairbond to the flock or to potential rivals (Mausz et al. 1992).

Faecal Hormone Analysis

To avoid handling of the birds, faecal samples instead of blood samples were used to monitor T and B. Thus, plasma levels of hormones can only be inferred. A parallel excretion study with domestic geese has shown significant correlations between plasma steroids and metabolites in the faeces. In particular, steroid surges in the plasma caused by challenges are accurately reflected in the faeces (Hirschenhauser 1998). Therefore, faecal samples are assumed to represent an integrated, proportional record of the plasma steroid levels within approximately 1–2 h prior to defecation (Krawany 1996). Individual faecal samples were collected within 2 h following behavioural observations and stored at -20°C until analysis. A total of 202 faecal samples were collected from the 37 males throughout the sampling period. Between 3 and 22 faecal samples per individual were analysed.

Faecal T and B equivalents (Krawany 1996) were extracted at a methanol dilution of 1:150, hydrolysed using a mixture of β -glucuronidase/arylsulfatase (Merck 4114), and determined by enzyme immunoassay (EIA) using group-specific antibodies as described elsewhere (Kotrschal et al. 1998; Hirschenhauser et al. 1999a,b). The sensitivity of the assay was less than 2 pg/well, and concentration limits for reliable measurement ranged from 0.5 to 44.4 ng g^{-1} faeces for T and

from 0.4 to 96.7 ng g⁻¹ faeces for B. The intra-assay coefficient of variation was 9.8% for T and 12.3% for B, and the interassay coefficient of variation was 14.7% for T and 16.9% for B.

Data Processing and Statistics

The sampling period was divided into the following six phases: (a) 'Feb.', the beginning of Feb. until 4 wk before the first egg (n = 11 males); (b) 'early mating', 4–2 wk before the first egg (n = 8); (c) 'pre-laying', 2 wk before the first egg (n = 13); (d) 'laying', from the day the first egg was laid until initiation of incubation (n = 14); (e) 'incubation', the nesting period, when only the female incubates the clutch (n = 6); (f) 'hatching', from hatch until the goslings were 2 wk old (n = 3). During later nesting phases, when a pair had (g) 'lost the clutch' (n = 9) or a male had (h) 'lost his partner' to the challenging competitor (n = 14), we plotted the data as additional events along the *x*-axis. For unpaired challengers, data were analysed according to the timing of the challenged pair involved. Only the 'Feb.' and the 'lost partner' phases include males that were not sampled in multiple phases; however, this is appropriate because, in earlier studies, the seasonal patterns of T and B during mating phases did not vary significantly between males of the same social category (Kotrschal et al. 1998; Hirschenhauser et al. 1999b).

For different levels of comparison, three different means were generated. We calculated means per individual per phase (n males in Fig. 1) to assess seasonal fluctuations and phasewise covariations between hormonal and behavioural parameters. Individual means over the entire sampling period (n = 37 males) served to scan for correlations between hormones and behaviour throughout the entire reproductive season. For comparison between male categories, we plotted and correlated these individual means of hormones and behaviour over the entire sampling period for each of the social categories separately (n males in Fig. 2).

To avoid problems with data distribution, we used non-parametric statistical procedures. For comparison of seasonal hormone fluctuations throughout the sampling period we employed Friedman and Wilcoxon matched-pairs tests. To compare faecal hormones and behaviour between the male categories we used Kruskal–Wallis and Mann–Whitney U-tests with posthoc Bonferroni correction. Covariations between hormones and behaviour we calculated with Spearman's rank correlation. If two correlated parameters were both significantly correlated with an additional third parameter, we only considered these correlations significant when they remained at $p < 0.05$ after partial correlation. This test is controlling for redundant effects of the intercorrelation between three or more variables (Zöfel 1992). All probabilities given are two-tailed.

Results

Seasonal Patterns of Hormones

Faecal T levels were high during the mating and pre-laying phases and dropped towards egg-laying (Wilcoxon test: $Z_8 = -2.10$; $p = 0.036$). Another significant

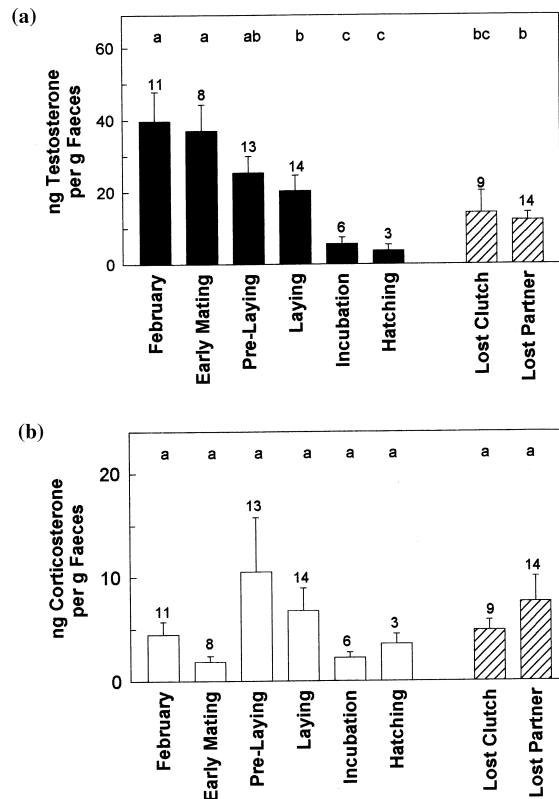


Fig. 1: Seasonal pattern of faecal (a) testosterone (T; black bars) and (b) corticosterone (B; open bars) equivalents (mean per phase \pm SE) in all males sampled throughout the reproductive season. Numbers above bars indicate sample sizes (n individuals). Phases along the x -axis are explained in the Methods section. Additionally, we plotted mean T and B (\pm SE) of males from pairs which, during later phases, had lost their clutch and males who had lost their partner to a challenging competitor (hatched bars). Significant differences between phases (Friedman and Wilcoxon test) are indicated by different letters (a, b, c)

decrease in T was found in ganders whose females had initiated incubation (Wilcoxon test: $Z_6 = -1.99$; $p = 0.046$; Fig. 1a). T levels were increased in males who had lost their partners compared to males whose females were incubating (Mann-Whitney U-test: $Z_{14,6} = -2.06$; $p = 0.039$). However, these levels did not return to the high levels of the early mating phase (Mann-Whitney U-test: $Z_{14,8} = -2.73$; $p = 0.006$). The T of males whose female had lost the clutch was not significantly different from the T of males whose female was incubating (Mann-Whitney U-test: $Z_{9,6} = -0.24$; $p = 0.814$).

Little seasonal variation was found in the B values of males (Fig. 1b). The highest B was excreted during pre-laying and laying, whereas lowest B was found in males whose female had started incubation. However, these fluctuations did not

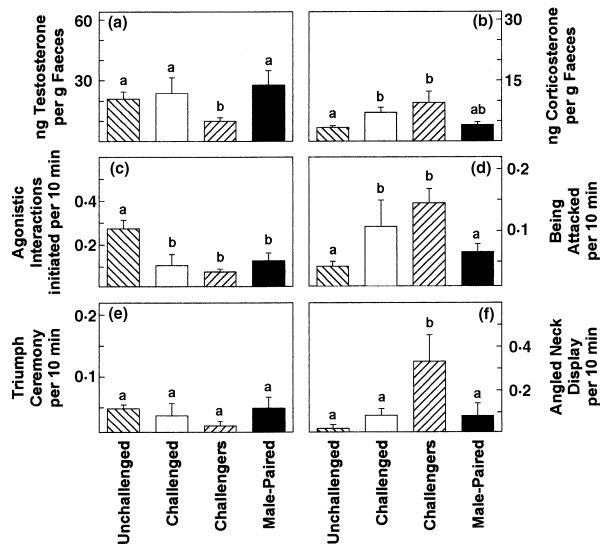


Fig. 2: Faecal (a) testosterone and (b) corticosterone equivalents and frequencies of (c) agonistic male-male interaction initiated, for (d) being the recipient of an attack, (e) Triumph Ceremony and (f) Angled Neck display per 10 min observation in the male social categories. Plotted are $\bar{x} \pm \text{SE}$ per social category over the entire sampling period. Males were classified according to their role in conflicts over females as 'unchallenged males' ($n = 18$; hatched bars), 'challenged males' ($n = 6$; open bars), 'challengers' ($n = 6$; hatched bars) and 'male-paired males' ($n = 7$; black bars). Significant differences between social categories (Mann-Whitney U-test) are indicated by different letters (a, b)

vary significantly over the reproductive phases (Friedman test: $\chi^2 = 5.10$; $df = 5$; $p = 0.404$).

Hormones and Behaviour in Different Categories of Males

Over the entire sampling period, the challengers had marginally lower T than the challenged males (Mann-Whitney U-test: $Z_{6,6} = -2.08$; $p = 0.064$) or male-paired males (Mann-Whitney U-test: $Z_{6,7} = -2.14$; $p = 0.064$; Fig. 2a). In contrast, B was significantly higher in both male categories involved in challenging situations than in unchallenged males (Mann-Whitney U-test, challenged males: $Z_{6,18} = -2.53$; $p = 0.023$; challengers: $Z_{6,18} = -2.27$; $p = 0.047$; Fig. 2b), whereas B was not significantly different between challenged males and challengers (Mann-Whitney U-test: $Z_{6,6} = -0.64$; $p = 0.522$).

Agonistic male-male interactions were initiated more frequently by unchallenged males than by challengers (Mann-Whitney U-test: $Z_{6,18} = -2.77$; $p = 0.011$; Fig. 2c). In contrast, the challengers were attacked more often than unchallenged males (Mann-Whitney U-test: $Z_{6,18} = -3.41$; $p = 0.001$) or male-paired males (Mann-Whitney U-test: $Z_{6,7} = -2.30$; $p = 0.043$; Fig. 2d). Also, the challengers displayed Angled Neck more frequently than any other male category (Mann-Whitney U-test, unchallenged males: $Z_{6,18} = -3.68$; $p < 0.000$; male-

paired males: $Z_{6,7} = -2.20$; $p = 0.055$; Fig. 2f). Frequencies of Triumph Ceremonies did not differ between the male categories (Kruskal Wallis test: $\chi^2 = 5.00$; $df = 3$; $p = 0.172$; Fig. 2e).

Correlations between Hormones and Behaviour

Hormones and male–male interactions

We found no significant correlations between T and B (Table 1a–c). The individual means of all males sampled over the entire sampling period did not show a correlation between agonistic male–male interactions and T (Table 1a). This was also true for correlations within each social category (Table 1b). During laying, but not during any other phase of the sampling period, agonistic frequencies were positively correlated with T (Table 1c; Fig. 3).

Throughout the entire reproductive season, excreted amounts of B equivalents scaled positively with frequencies of being attacked by other flock members (Table 1a). Comparing social categories, the positive correlation between frequencies of being attacked and mean faecal B was found only in the challengers (Table 1b), whereas within unchallenged males (Spearman's rank correlation: $r_s = 0.01$; $n = 18$; $p = 0.974$), challenged males (Table 1b) and male-paired males ($r_s = 0.76$; $n = 7$; $p = 0.056$), we found no significant correlations (Fig. 4b). However, in males who had lost their partner (Fig. 1), excreted B correlated significantly with frequency of being attacked ($r_s = 0.68$; $n = 13$; $p = 0.011$; Fig. 4a).

Hormones and male–female interactions

Faecal T and the frequency of Triumph Ceremony were not significantly correlated when all males were considered over the entire sampling period or within social categories (Table 1a–b). During the laying phase, however, males with higher levels of faecal T performed Triumph Ceremonies more often. In contrast, during the pre-laying phase this correlation was not significant (Table 1c; Fig. 5).

Because the Triumph Ceremony is always started with an attack, a positive correlation between aggressiveness and Triumph Ceremony could be expected. In all males over the entire sampling period there was a significant correlation between the two parameters (Table 1a), but this correlation did not hold on the level of social categories (Table 1b). In addition it was not significant within the different phases (Table 1c). Only in males from pairs that had lost the clutch (Fig. 1) were frequencies of agonistic interaction and of Triumph Ceremony significantly positively correlated ($r_s = 0.98$; $n = 6$; $p < 0.0001$). Neither of the two behaviours was significantly correlated with T in these males (agonistic interaction: $r_s = 0.28$; $n = 6$; $p = 0.594$; Triumph Ceremony: $r_s = 0.21$; $n = 6$; $p = 0.686$).

In all males sampled throughout the reproductive season, mean faecal B and frequencies of Angled Neck were not significantly correlated (Table 1a). However, this correlation was significantly positive during egg-laying (Table 1c). No significant correlation between B and Angled Neck rate was found in the males who

Table 1: (a) Spearman's rank correlation coefficients between individual means of faecal testosterone (T), corticosterone (B) and behavioural parameters over the entire sampling period in all males (n = 37); (b) within the challenged males (n = 6; above right) and within the challengers (n = 6; bottom left); (c) in all males sampled during the pre-laying phase (n = 12; above right) and during laying (n = 11; bottom left)

	T	B	Agonistic interaction	Being attacked	Triumph Ceremony	Angled Neck display
(a)						
B	$r_s = 0.17$ ns					
Agonistic interaction	$r_s = 0.30$ $p = 0.076$	$r_s = -0.19$ ns				
Being attacked	$r_s = -0.19$ ns	$r_s = 0.56$ $p < 0.0001$	$r_s = -0.52$ $p = 0.001$			
Triumph Ceremony	$r_s = 0.35$ $p = 0.036^*$	$r_s = -0.09$ ns	$r_s = 0.47$ $p = 0.003$	$r_s = -0.37$ $p = 0.023$		
Angled Neck display	$r_s = -0.15$ ns	$r_s = 0.38$ $p = 0.021^{**}$	$r_s = -0.26$ ns	$r_s = 0.38$ $p = 0.021$	$r_s = -0.05$ ns	
(b)						
T	—	$r_s = 0.83$ $p = 0.042^a$	$r_s = -0.18$ ns	$r_s = 0.94$ $p = 0.005$	$r_s = 0.06$ ns	$r_s = 0.09$ ns
B	$r_s = 0.66$ ns	—	$r_s = 0.09$ ns	$r_s = 0.77$ $p = 0.072$	$r_s = 0.41$ ns	$r_s = 0.44$ ns
Agonistic interaction	$r_s = 0.20$ ns	$r_s = 0.20$ ns	—	$r_s = -0.27$ ns	$r_s = 0.54$ ns	$r_s = 0.86$ $p = 0.027$
Being attacked	$r_s = 0.43$ ns	$r_s = 0.94$ $p = 0.005$	$r_s = 0.06$ ns	—	$r_s = 0.12$ ns	$r_s = -0.09$ ns
Triumph Ceremony	$r_s = -0.09$ ns	$r_s = 0.52$ ns	$r_s = 0.40$ ns	$r_s = 0.67$ ns	—	$r_s = 0.63$ ns
Angled Neck display	$r_s = -0.60$ ns	$r_s = -0.77$ ns	$r_s = -0.23$ ns	$r_s = -0.60$ ns	$r_s = -0.03$ ns	—
(c)						
T	—	$r_s = 0.38$ ns	$r_s = -0.54$ ns	$r_s = 0.19$ ns	$r_s = -0.24$ ns	$r_s = 0.12$ ns
B	$r_s = -0.01$ ns	—	$r_s = -0.09$ ns	$r_s = 0.56$ ns	$r_s = -0.06$ ns	$r_s = 0.65$ ns
Agonistic interaction	$r_s = 0.47^b$ ns	$r_s = 0.11$ ns	—	$r_s = -0.10$ ns	$r_s = 0.30$ ns	$r_s = 0.28$ ns
Being attacked	$r_s = 0.02$ ns	$r_s = 0.23$ ns	$r_s = -0.08$ ns	—	$r_s = 0.19$ ns	$r_s = 0.62$ $p = 0.034$
Triumph Ceremony	$r_s = 0.64$ $p = 0.035$	$r_s = -0.02$ ns	$r_s = 0.31$ ns	$r_s = -0.13$ ns	—	$r_s = 0.20$ ns
Angled Neck display	$r_s = -0.04$ ns	$r_s = 0.83$ $p = 0.002$	$r_s = -0.08$ ns	$r_s = 0.36$ ns	$r_s = -0.07$ ns	—

* $p > 0.05$ (ns) when controlled for the intercorrelation with frequencies of agonistic interactions using partial correlation.

** $p > 0.05$ (ns) when controlled for the intercorrelation with frequencies of being attacked.

^a $p > 0.05$ (ns) when controlled for the intercorrelation with frequencies of being attacked.

^b After partial correlation controlling for triumph frequencies: $r = 0.70$; $df = 8$; $p = 0.025$.

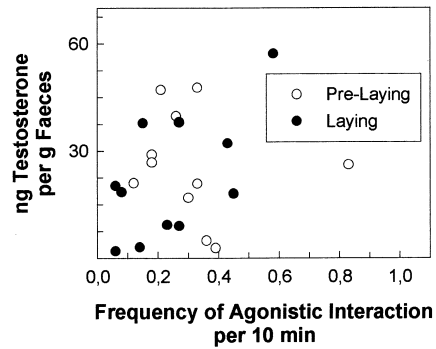


Fig. 3: The relationship between frequencies of agonistic male–male interactions initiated per 10 min and faecal testosterone in the pre-laying phase (Spearman's rank correlation: $r_s = -0.54$; $n = 11$; $p = 0.778$; open dots) and during laying (partial correlation: $r = 0.70$; $df = 8$; $p = 0.025$; Table 1c; black dots). Plotted are individual means per phase

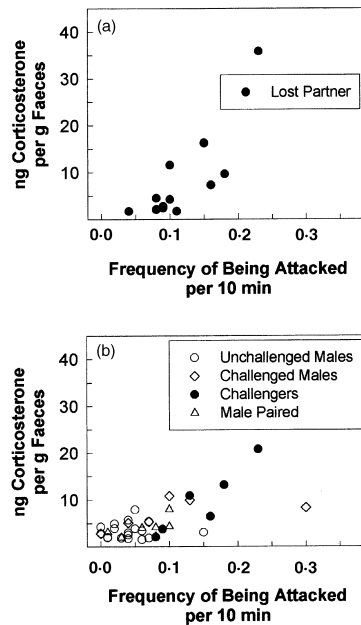


Fig. 4: The relationship between frequencies of being attacked per 10 min and faecal corticosterone (B). Plotted are (a) individual means in males who had lost their partner (Spearman's rank correlation: $r_s = 0.68$; $n = 13$; $p = 0.011$) and (b) individual means over the entire sampling period for the different social categories. (Unchallenged males: $r_s = 0.01$; $n = 18$; $p = 0.974$; open dots. Challenged males: $r_s = 0.77$; $n = 6$; $p = 0.072$; diamonds. Challengers: $r_s = 0.94$; $n = 6$; $p = 0.005$; black dots. Male-paired males: $r_s = 0.76$; $n = 7$; $p = 0.046$; triangles.)

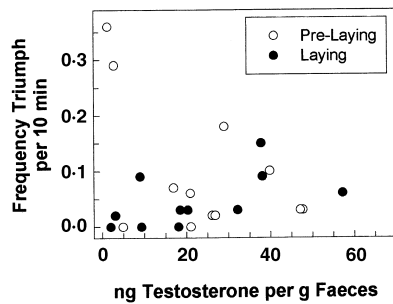


Fig. 5: The relationship between faecal testosterone (T) and Triumph Ceremony rates per 10 min in the pre-laying phase (Spearman's rank correlation: $r_s = -0.24$; $n = 12$; $p = 0.461$; open dots) and during laying ($r_s = 0.64$; $n = 11$; $p = 0.035$; black dots). Plotted are individual means per phase

had lost their partner ($r_s = 0.07$; $n = 11$; $p = 0.824$), or within any of the social categories (Table 1b).

Discussion

In accordance with the challenge hypothesis (Wingfield et al. 1990), androgens are involved in reproductively directed agonistic interactions rather than in other forms of aggression. Therefore, it was not surprising that testosterone (T) and general levels of agonistic behaviour were not always correlated. During the mating phases circulating T is at its maximum (Hirschenhauser et al. 1997, 1999a,b), because physiology is dominated by endocrine factors associated with spermatogenesis and copulation (Bluhm et al. 1984; Dittami & Reyer 1984; Ramenofsky 1984). Therefore, T may not respond to behavioural stimulation, such as male–male interactions (Wingfield et al. 1990).

Exclusively in the laying phase, when T is seasonally decreased after its peak at mating, agonistic interactions and Triumph Ceremonies were significantly correlated with faecal T. During egg-laying, agonistic male–male interactions may still occur at high frequencies (Lorenz 1974; Akesson & Raveling 1984; Lamprecht 1987) and we expect that the laying greylag female will be intensively guarded by her male partner to ensure paternity of the clutch (Moore 1984; Lamprecht 1987; Ely 1989; Birkhead & Møller 1993). Thus, the responsiveness of male T during the egg-laying phase is functionally plausible and can be elicited and measured, because male T levels at laying are no longer masked by seasonal maxima.

Only in males from pairs that had lost their clutch, but not in any other social category, a positive correlation between rates of agonistic male–male interactions and frequencies of Triumph Ceremony was observed. In these males, intense Triumph activities may have been related to possible attempts to produce a replacement clutch late in the season (Owen & Black 1990; Cooke et al. 1995).

Frequencies of agonistic interactions in the challenged males covaried with elevated T and corticosterone (B) levels, but T and B were not correlated with one

another. We found the highest frequencies of attacking in unchallenged males, while these were only rarely the recipients of attacks. Accordingly, these males had comparatively high faecal T, but their B was low. In contrast, and contrary to our expectations (Wingfield et al. 1990), the challengers were high in B but were low in T. Following our sampling period, none of the unpaired challengers succeeded in winning the female that he was competing for. Thus, the low levels of T may reflect their 'losing' the contest with high rates of being attacked and low rates of initiated aggressive behaviour. In baboons *Papio anubis*, for example, T profiles were lower in low-ranking and low-aggressive than in high-ranking and high-aggressive males during socially unstable situations (Sapolsky 1983). In contrast to the losing challengers, our challenged males were high in T (Fig. 2a), comparable to male song sparrows *Melospiza melodia*, which had increased T when challenged by a conspecific male (Wingfield & Wada 1989).

However, by signalling his interest towards an already paired female, a challenger risked being chased by higher ranking (paired) individuals, which increased B in this male. We suggest that the high levels of excreted B in unpaired challenging males may be the result of a social conflict in these individuals, which is characterized by his attempts to win a paired female and, at the same time, losing agonistic interactions with her mate. This conflict may reflect the trade-off between signalling to a potential mate, while, at the same time, increasing the risk of being attacked by a rival (Sullivan 1994).

The Triumph Ceremony is a more conspicuous signal than the Angled Neck display. Triumph Ceremonies may not exclusively be addressed to the female but also to the rest of the flock (Mausz et al. 1992). A female might prefer males on the basis of conspicuous, potentially costly signals (Zahavi 1975). When high rates of being attacked and excreted B are considered as costs, the Angled Neck display may also be regarded as a costly signal for an unpaired challenger. None of our challengers succeeded. The lack of a positive female response to the Angled Neck display of the challengers may explain why they did not perform the Triumph Ceremony in most cases.

T and B patterns were similar in male-paired males and in heterosexually paired, unchallenged males. Thus, heterosexually and homosexually paired ganders were hormonally indistinguishable. This was in some contrast to the behavioural patterns. Male-paired males had comparatively low frequencies of being attacked. However, in this study both heterosexually and homosexually paired males had higher levels of T than the unpaired challengers. Therefore, as was shown in an earlier study (Hirschenhauser et al. 1999b), T levels were related to pairbond status rather than to social rank *per se*.

In summary, our results confirm the expectation that in male greylag geese interactions between androgens and behaviour shift quantitatively between the different phases of the reproductive season. Only during laying, at the onset of seasonally decreasing T and when paternity might be at stake, were agonistic male–male interactions significantly correlated with T. The conflict of the challengers between courting a paired female and high rates of being attacked by the challenged male was reflected in high levels of excreted B. Thus, pairbond status and social

conflict in interaction with the seasonally varying social functions modulate individual T and B.

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