

Didone Frigerio · Katharina Hirschenhauser ·
Erich Möstl · John Dittami · Kurt Kotrschal

Experimentally elevated testosterone increases status signalling in male Greylag geese (*Anser anser*)

Received: 2 January 2003 / Revised: 5 April 2004 / Accepted: 19 April 2004 / Published online: 15 July 2004
© Springer-Verlag and ISPA 2004

Abstract Testosterone modulates male vertebrates' sexual and social behaviour. We experimentally investigated the testosterone-sensitive behaviours in male greylag geese (*Anser anser*) by implanting silastic tubes containing crystalline testosterone during the mating season (February; 5 implanted and 5 control males) and in the early winter (November; 7 and 7). Focal animals were part of a semi-tame, unrestrained flock with fully intact social relationships. Excreted testosterone and corticosterone immunoreactive metabolites (TM, BM) were determined by enzyme immunoassay. Individual faecal samples and behavioural protocols were collected daily over a period of 5 weeks, including 1 control week before implantation. In February, no significant behavioural effects of the supplemental testosterone were observed, which may be due to the naturally occurring high systemic androgen levels in spring. In November, however, implanted males had higher TM excretion rates and performed status signalling behaviour ("beak up") more frequently than control males. No differences between implanted and control males were found with respect to BM, agonistic interactions or vigilance behaviour. Furthermore, during the second week after implantation, TM positively correlated with the frequency of "beak up" of implanted males, whilst their female partners were attacked with lower latency by other members of the flock than the females of control males. Hence, status signalling in greylag ganders seems

to be testosterone-sensitive year-long and "inappropriate" status signalling of males may draw attacks towards their females.

Keywords *Anser anser* · Corticosterone · Faeces · Status signalling behaviour · Testosterone implantation

Introduction

Gonadal steroid hormones modulate sexual and agonistic behaviour in vertebrates (e.g. fishes: Oliveira et al. 1996; amphibians: Houck and Woodley 1995; reptilians: Moore and Thompson 1990; birds: Wingfield et al. 1990; or mammals: Woodroffe et al. 1997). Behaviour, in turn, can produce feed-back modulation on the endocrine system (e.g. Hegner and Wingfield 1986; Wingfield et al. 1990; Knapp and Moore 1996; Wikelski et al. 1999). In particular, studies on male birds have shown that testosterone (T) is involved in modulating sexual and social behaviours, for example by mediating the trade-off between sexual and parental investment (e.g. Rissman and Wingfield 1984; Ketterson and Nolan 1992; Wikelski et al. 1999). High T levels may also promote acoustic communication (e.g. Ketterson et al. 1992; Hunt et al. 1997) and further social status (e.g. Searcy and Wingfield 1980; Hoysak and Ankney 1996).

Experimentally increased systemic T is known to change the level of expression of androgen-sensitive behaviours, for example by promoting polygynous mating (e.g. Hegner and Wingfield 1987; Ketterson and Nolan 1992), inhibiting paternal care (e.g. Dittami et al. 1991; Cawthorn et al. 1998) or facilitating territoriality (e.g. Wingfield and Hahn 1994). However, elevated T may also impose costs by suppressing immuno-competence and increasing mortality (e.g. Saino et al. 1995; Ros 1999; Wingfield et al. 1999) or by delaying moult (e.g. Schleussner et al. 1985).

Although many studies demonstrated that T secretion in spring was related to the expression of aggressive behaviour (e.g. Wingfield and Ramenofsky 1985; Wing-

Communicated by R.F. Oliveira

D. Frigerio (✉) · K. Hirschenhauser · J. Dittami · K. Kotrschal
Konrad Lorenz Forschungsstelle für Ethologie and Department
of Zoology, University of Vienna,
Grünau 11,
4645 Vienna, Austria
e-mail: klf.gruenau@telecom.at
Tel.: +43-7616-8510
Fax: +43-7616-85104

E. Möstl
Institute for Biochemistry, University of Veterinary Medicine,
Vienna, Austria

field et al. 1990; Canoine and Gwinner 2002), aggression and T are not always linked. Some temperate-zone birds, for example, show aggressive and territorial behaviour on their wintering grounds, when gonads are regressed and plasma T is low (e.g. Silverin 1980; Schwabl 1992; Gwinner et al. 1994). Furthermore, a seasonally dependent androgen sensitivity was proposed for behaviours, such as mate guarding and sexually dimorphic vigilance, which seem to be T-dependent only during the reproductive season (Saino and Møller 1995; Fusani et al. 1997). However, in geese of both sexes, frequencies of agonistic interactions co-vary with testosterone only outside the breeding season (Dittami and Reyer 1984). Hence, T may modulate aggression and other behaviours in a seasonally dependent and species-specific context.

To investigate T-dependent male behaviours in greylag geese (*Anser anser*), we compared the behavioural effects of experimentally enhanced T levels during the mating season in February and during the early winter flock situation in autumn (November). The behaviour of implanted and control males living in a natural flock context was monitored and the amounts of excreted testosterone and corticosterone immunoreactive metabolites (TM, BM) were measured from faeces by enzyme immunoassay (EIA; Krawany 1996). Implanted males were expected to excrete higher concentrations of TM, to be more aggressive and to perform a particular vigilance-related, social status signalling behaviour (“beak up”; Lazarus and Inglis 1978; Lorenz 1988; Waldenberger and Kotschal 1993) more frequently than their controls. Furthermore, as altered T-dependent behaviours may increase social stress (e.g. Sorenson et al. 1997; Schoech et al. 1999), the social interactions of implanted individuals with other flock members as well as their corticosterone levels may be affected. As the pairbond in geese is continuous over the year (i.e. not restricted to the reproductive season) and pairs act as social unit within the flock, we expected the social interactions of the female partners of the implanted males to be affected too.

Methods

Animals

The studied non-migratory flock of greylag geese was introduced into the Upper-Austrian valley of the river Alm by Konrad Lorenz and co-workers in 1973 (Lorenz 1988). The geese are unrestrained and generally spend the days close to the research station where they are provided with supplemental food twice a day year-round. At night, the birds roost on a nearby lake (Almsee). Natural predators (mainly red foxes and golden eagles) take approximately 10% of the population per year (Hemetsberger 2001). All individuals are marked with coloured leg rings and are habituated to the close presence of humans. Social behaviour and individual life-history have been monitored since 1973. Approximately 20% of the flock members are carefully hand-raised.

The flock consisted of 124 individuals in February 1996 and 133 in November 1998. The present study is based on 24 adult males (Table 1); 12 of them were implanted (Im) with T and 12 were used as untreated controls (Co) in two identical experiments in two different seasons. During the first experiment, in February 1996, ten males ($n_{1Im}=5$ implanted and $n_{1Co}=5$ controls) of different age (age-range implanted: 3–19 years; age-range controls: 3–23 years; mean±SD = 10.7±7.7) and from different social categories (6 unpaired, 4 paired without offspring) were considered (Table 1). During the second season, in November 1998, 14 paired males without offspring ($n_{2Im}=7$ implanted and $n_{2Co}=7$ controls) of different age (age-range implanted: 2–11 years; age-range controls: 3–13 years; mean±SD = 6.2±3.2) were used (Table 1). Sample size was constrained by our ability to catch all of those unrestrained males within a single day, which was necessary to ensure synchrony between implanted individuals.

Implantation

In accordance with the annual pattern of excreted testosterone metabolites (TM; Hirschenhauser et al. 1999a), T levels were manipulated in two different seasons: (1) at the

Table 1 List of the focal male Greylag Geese (*Anser anser*) (Im implanted male, Co control male) considered in each experimental situation (season 1 = mating, February 1996; season 2 = early winter, November 1998), their age and social status at the time of data collection. Behaviour of female partners of paired males was also sampled

Individual <i>N</i>	Season	Age (years)	Social status
1-Im	1	10	Unpaired
2-Im	1	3	Paired
3-Im	1	3	Paired
4-Im	1	19	Unpaired
5-Im	1	12	Unpaired
6-Co	1	3	Paired
7-Co	1	20	Unpaired
8-Co	1	23	Unpaired
9-Co	1	4	Unpaired
10-Co	1	10	Paired
11-Im	2	8	Paired
12-Im	2	11	Paired
13-Im	2	5	Paired
14-Im	2	5	Paired
15-Im	2	8	Paired
16-Im	2	2	Paired
17-Im	2	2	Paired
18-Co	2	13	Paired
19-Co	2	6	Paired
20-Co	2	6	Paired
21-Co	2	4	Paired
22-Co	2	8	Paired
23-Co	2	3	Paired
24-Co	2	6	Paired

beginning of the mating season (February 1996), when levels of TM are at maximum; and (2) during the winter flock situation (November 1998) when levels of TM are low. Silastic tubes (length 40 mm; i.d. 0.48 mm; o.d. 1.143 mm) were filled with crystalline T and sealed with silicon glue at the ends. Twelve ($n_{1lm} = 5 + n_{2lm} = 7$; Table 1) males were implanted with T-filled silastic tubes at the inner side of the right thigh. The implantation procedure involved local anaesthesia with chlorethyllic spray, feather removal, small cutting into the skin with a bistoury, subcutaneous implantation and a final disinfection. The whole procedure took less than 2 min per individual. Geese were caught by hand just before being implanted. For ethical reasons, and to keep the disturbance of the flock at a necessary minimum, we decided not to sham-implant the control males. However, in similar studies no significant differences were ever observed between sham-implanted controls and non-implanted controls (e.g. Dufty 1989; Saino and Møller 1995; Saino et al. 1995). We are therefore confident that the presented results are due to T effects rather than to the catching and holding procedure in animals that are occasionally caught and handled anyway (e.g. for ringing or weighing). As removal would have meant additional stress, implants were not removed after the experiments. However, as the tubes are tiny compared to the size of a goose, they did not seem to produce any adverse effect on the animal.

Data collection

In both experimental periods, individual behavioural protocols and faecal samples were collected over 5 consecutive weeks, including 1 control week before the implantation. Behavioural data were collected with a Psion Organiser and The Observer 2.0 software (Noldus Information Technology, Wageningen, Netherlands). Behavioural protocols consisted of 5 min continuous sampling (Martin and Bateson 1986) of the focal male and its partner, if present (Table 1). To standardise data collection, observations started after all the food was distributed and ended when the focal individuals began to rest after feeding, i.e. approximately from 0800 to 0900 hours, and from 1530 to 1600 hours. Times varied slightly according to seasonal differences in light regime and activity of the geese. During data collection, the observer (D.F.) stood at the edge of the feeding area, approximately 5 m from the focal individual or pair. On average, 44.2 ± 7.4 (mean \pm SD) protocols per individual were collected during the mating season (i.e. February 1996; total $n_1=442$) and 11.4 ± 0.9 (mean \pm SD) during the early winter flock (i.e. November 1998; total $n_2=160$).

Three behavioural categories have been considered: (1) status signalling display, described as “beak up” in geese (Lazarus and Inglis 1978; Lorenz 1988; Waldenberger and Kotschal 1993); (2) vigilance behaviour, including “head up” and “extreme head up” (Lazarus 1978; Lazarus and Inglis 1978; Lorenz 1988); and (3) agonistic interactions, such as threats, attacks or fights (Raveling 1970; Lorenz

1988), distinguishing the passive or active role of the focal male. The short duration of each of these events and their recognisable onsets and ends allowed this form of data collection. Therefore, the frequency of occurrence per 5-min protocols was considered for behavioural categories (1) and (2). For the third category, the latency was considered (starting from the end of the food distribution, i.e. the beginning of the observation period) and it was recorded as a measure for aggressive motivation. The difference between the latency of initiated agonistic events and the latency of received aggression was then determined. This parameter may provide information about how fast an individual responded to a challenge and whether it was faster in attacking or in being attacked. The status signalling display was only recorded for the males since it is hardly ever observed in females (Lorenz 1988; own unpublished observations).

For the assessment of excreted corticosterone and testosterone immunoreactive metabolites (BM, TM), faecal samples were collected from the resting individuals approximately 90–180 min after the morning observations. The time span corresponds to the expected peak of steroid excretion following a physiological or behavioural challenge in geese (Krawany 1996; Hirschenhauser et al. 2000a; Kotschal et al. 2000). Sampling was timed to avoid the early morning peaks of adrenal activity (Schütz et al. 1997; Rich and Romero 2001; Carere et al. 2003). Samples were frozen at -20°C within 1 h of collection, which avoided any changes in the faecal steroid contents (Hirschenhauser 1998; own unpublished data). On average 32.4 ± 1.6 (mean \pm SD) samples per individual were collected during the mating season (i.e. February 1996; total $n_1=324$) and 24.3 ± 1.3 (mean \pm SD) during the second experiment (i.e. November 1998; total $n_2=340$). Faeces were collected from males only, therefore levels of BM and TM are not known for the females.

Since repeated and regular collection of plasma in the field was neither feasible nor advisable because of the potential stress induction (Miller et al. 1991), plasma levels are unknown. However, in agreement with other studies (Bishop and Hall 1991; Cockrem and Rounce 1994; Palme et al. 1996), experiments with domestic geese showed deterministic relationships between plasma steroids and metabolites in the faeces (Hirschenhauser et al. 2000a; Kotschal et al. 2000). Therefore, we are confident that our faecal BM and TM levels are representative for plasma corticosterone and T within 2–3 h before defecation.

Faecal steroid assays

Faecal samples (0.5 g) were extracted in methanol, hydrolysed and both, excreted corticosterone and testosterone immunoreactive metabolites were assayed by enzyme immunoassay (EIA; Möstl et al. 1987). Details of the procedure and cross-reactivities are published elsewhere (corticosterone: Kotschal et al. 1998; testosterone: Hirschenhauser et al. 1999b). Specifically, the assay

used for testosterone has cross-reactions with other 17β -OH-androgens, as for example DHT. As testosterone and corticosterone are not present in the samples, the substances detected by the assays are indicated as testosterone or corticosterone immunoreactive metabolites and indicated, respectively, with TM and BM. Concentration limits for reliable measurements ranged from 0.45 ng/g to 112.2 ng/g for BM and from 0.12 ng/g to 45.8 ng/g for TM. Intra-assay and inter-assay coefficients of variations were determined from homogenised pool samples. Mean intra-assay coefficient of variation was 13.8% for BM and 9.1% for TM; mean inter-assay coefficient of variation was 15.7% for BM and 13.1% for TM. These values are in the typical range for EIA procedures with faeces, because the number of procedure steps involved increases total variation.

Data analysis

Data were analysed using the SPSS statistical program (Pfeifer 1991). Results of all tests are given two-tailed; levels of significance were corrected according to Bonferroni post hoc test whenever necessary (Rice 1989). Both, hormonal and behavioural data were not normally distributed (Kolmogorov–Smirnov: BM, $Z=4.53$, $n=663$, $P<0.0001$; TM, $Z=3.37$, $n=664$, $P<0.0001$; beak up-frequency, $Z=12.7$, $n=958$, $P<0.0001$; vigilance-frequency, $Z=5.89$, $n=958$, $P<0.0001$; agonistic interaction-

latency, $Z=7.07$, $n=958$, $P<0.0001$). To approach normal distribution, a logarithmic ($\ln x$) transformation was performed for hormone values as well as for behavioural data, where $\ln(x+1)$ was used to transform the values of frequency of “beak up” and vigilance, while $\ln(x+299)$ was used to transform the values of latency of agonistic interactions, in order to avoid to calculate the logarithm of zero (Zöfel 1992).

The individual mean value of TM, BM and behaviours per week was taken into further analysis. One-way ANOVA was performed to investigate the effects of implantation on TM, BM and behaviours, as well as the temporal patterns and the seasonal differences within male categories. The same test was also performed to investigate a possible age-effect within the male categories. As testosterone-sensitive behaviours were the focus of the experiment, only correlations between TM and all behavioural parameters were calculated using parametric Pearson correlation coefficients.

Since the number of females associated with the focal males ($n_{1\text{Im},\text{Co}}=2,2$; Table 1) was too small to perform any statistics in the mating season (i.e. February 1996), comparisons between females were only possible in the second season (i.e. November 1998; $n_{2\text{Im},\text{Co}}=7,7$; Table 1) and a Student’s t -test was used on individual means per week.

Fig. 1 Patterns of **a,b** excreted testosterone immunoreactive metabolites (TM) and **c,d** excreted corticosterone immunoreactive metabolites (BM) in implanted (*black circles*; mean +SE) and control males (*open circles*; mean -SE) during the **a, c** mating season (February 1996; $n_{1\text{Im}}=5$, $n_{1\text{Co}}=5$ except the control week where $n_{1\text{Co}}=4$) and the **b,d** early winter experiment (November 1998; $n_{2\text{Im}}=7$, $N_{2\text{Co}}=7$). Data were collected 1 control week before the implantation (Co) and four consecutive weeks thereafter (first to fourth)

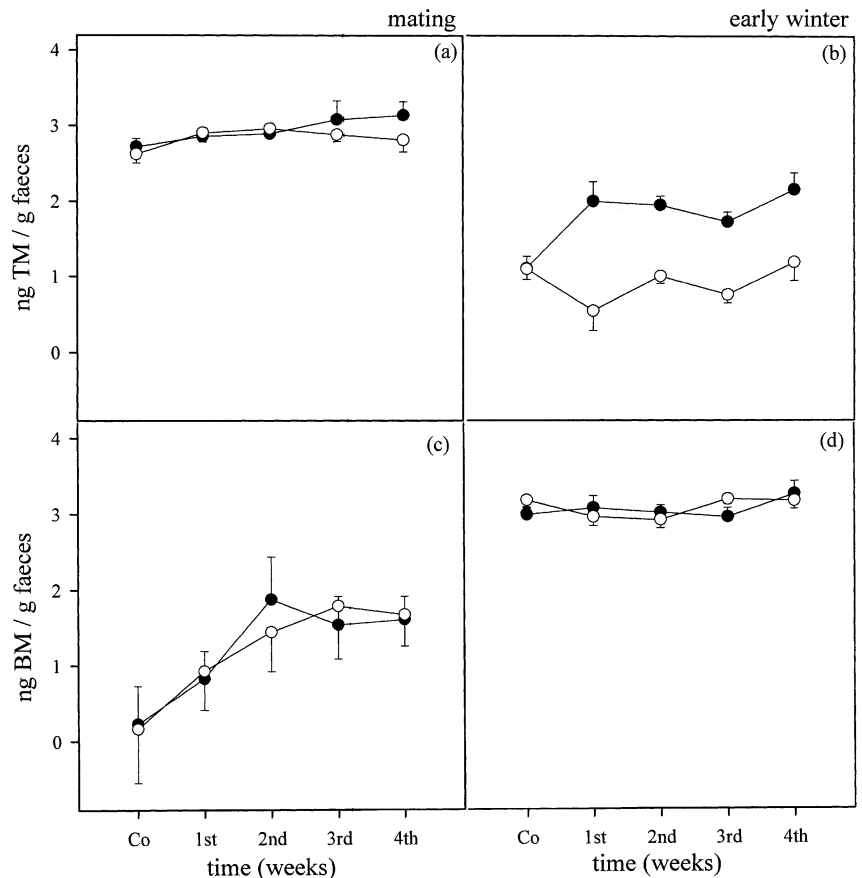


Table 2 Effects of implantation on excreted testosterone and corticosterone immunoreactive metabolites (TM, BM) and all behavioural parameters, frequency of “beak up”, frequency of vigilance and latency of agonistic interactions (BU-fr, Vig-fr, AI-lat) per season; the temporal patterns of TM, BM and behaviours, in implanted and control males in both seasons; the seasonal differences in TM, BM and behaviours, in implanted and control males. Significant results are in bold

	TM	BM	BU-fr	Vig-fr	AI-lat
Mating season (February)	$F_{1,48}=1.045, P=0.312$	$F_{1,48}=0.006, P=0.937$	$F_{1,48}=0.108, P=0.744$	$F_{1,48}=1.499, P=0.227$	$F_{1,48}=0.116, P=0.735$
Early winter (November)	$F_{1,69}=42.726, P<0.0001$	$F_{1,69}=0.108, P=0.743$	$F_{1,68}=14.097, P<0.0001$	$F_{1,68}=0.016, P=0.9$	$F_{1,68}=0.047, P=0.83$
Mating season (February)					
Im-males	$F_{4,24}=1.09, P=0.388$	$F_{4,24}=2.428, P=0.081$	$F_{4,24}=0.783, P=0.550$	$F_{4,24}=6.35, P=0.002$	$F_{4,24}=0.758, P=0.565$
Co-males	$F_{4,23}=1.06, P=0.403$	$F_{4,23}=1.231, P=0.331$	$F_{4,23}=0.17, P=0.951$	$F_{4,23}=3.948, P=0.017$	$F_{4,23}=1.436, P=0.261$
Early winter (November)					
Im-males	$F_{4,34}=4.863, P=0.004$	$F_{4,34}=0.848, P=0.506$	$F_{4,34}=4.521, P=0.006$	$F_{4,34}=0.218, P=0.926$	$F_{4,34}=0.612, P=0.657$
Co-males	$F_{4,34}=2.093, P=0.106$	$F_{4,34}=1.691, P=0.178$	$F_{4,33}=0.636, P=0.641$	$F_{4,33}=2.817, P=0.043$	$F_{4,33}=1.564, P=0.21$
Im-males	$F_{1,59}=72.08, P<0.0001$	$F_{1,59}=90.801, P<0.0001$	$F_{1,59}=0.041, P=0.84$	$F_{1,59}=9.84, P=0.003$	$F_{1,59}=3.67, P=0.06$
Co-males	$F_{1,58}=282.59, P<0.0001$	$F_{1,58}=68.475, P<0.0001$	$F_{1,58}=8.506, P=0.005$	$F_{1,57}=3.704, P=0.059$	$F_{1,58}=1.523, P=0.222$

Results

Mating season, February

No significant differences were found between the levels of excreted testosterone immunoreactive metabolites (TM) of implanted and control males (Table 2; Fig. 1a). The same is true for levels of excreted corticosterone metabolites (BM; Table 2; Fig. 1c) as well as for all behavioural parameters, frequency of beak up (Table 2; Fig. 2a), frequency of vigilance (Table 2; Fig. 2c) and latency of agonistic interactions (Table 2; Fig. 2e).

When considering the temporal patterns, no significant differences were found for TM or BM either among implanted or control males (Table 2; Fig. 1a,c). The same is true for two of the behavioural parameters considered, i.e. frequency of beak up and frequency of vigilance (Table 2; Fig. 2a,e). The temporal pattern of the frequency of vigilance was in fact significantly decreasing in both male categories (Table 2; Fig. 2c). Finally, an age-effect was detected ($F_{1,48}=6.265, P=0.016$).

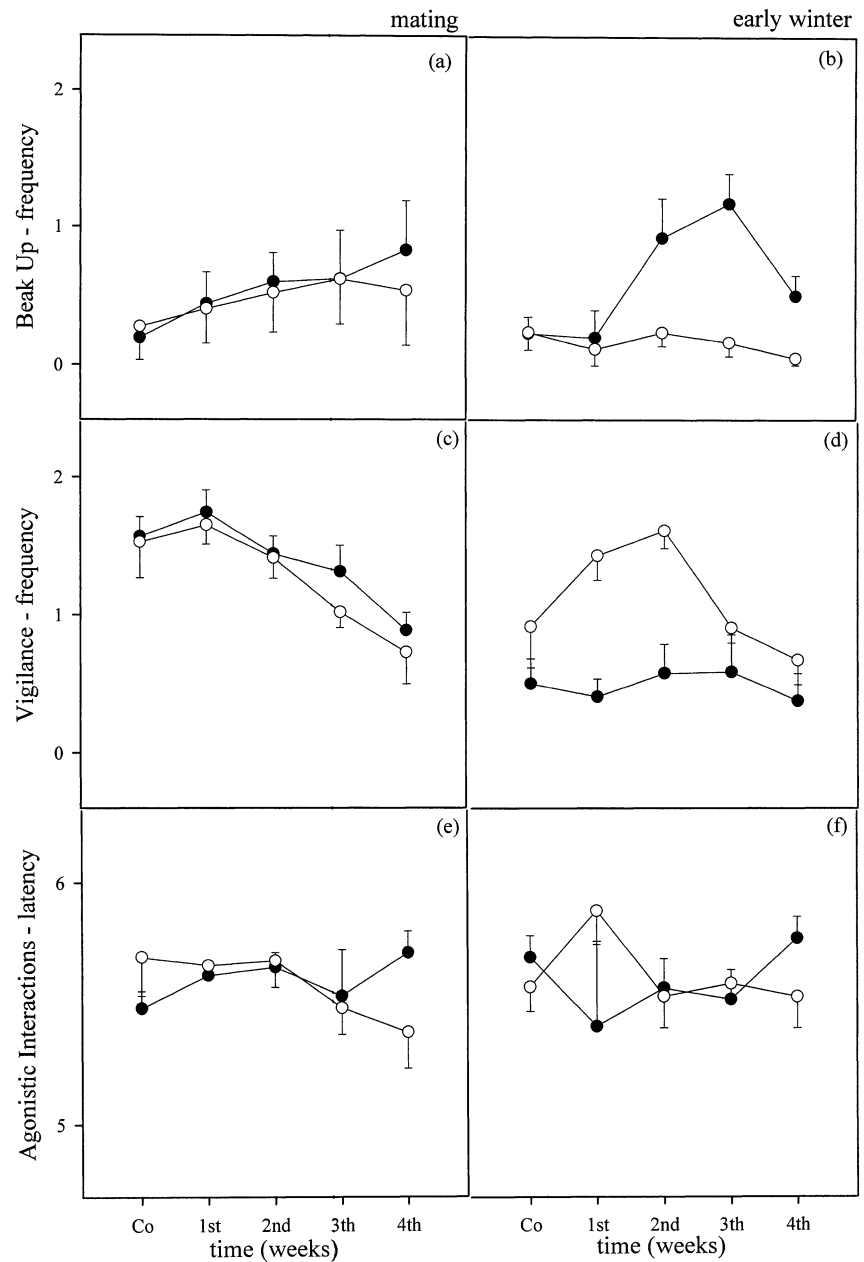
The implantation did not produce any significant hormonal or behavioural differences between male categories (Figs. 1a,c, 2a,c,e), therefore implanted and control males were plotted together in order to scan for correlations. However, no significant correlation was observed (TM and beak up: $r=-0.34, n=10, P=0.34$; TM and vigilance: $r=0.22, n=10, P=0.55$; TM and agonistic interactions: $r=-0.19, n=10, P=0.586$).

Early winter flock situation, November

In November, a significant difference was found between the levels of excreted testosterone immunoreactive metabolites (TM) of implanted and control males (Table 2; Fig. 1b), but not between the levels of excreted corticosterone immunoreactive metabolites (BM; Table 2; Fig. 1d). Significant differences were observed between implanted and control males in the frequency of “beak up” (Table 2; Fig. 2b), but not in the frequency of vigilance (Table 2; Fig. 2d), or in the latency of agonistic interactions (Table 2; Fig. 2f).

During the sampling period, TM significantly increased in implanted but not in control males (Table 2; Fig. 1b). Specifically, TM of implanted males increased already in the first week after implantation (Bonferroni post hoc test: control-week vs first week after: $P=0.02$; control-week vs second week after: $P=0.033$; control-week vs fourth week after: $P=0.004$; Fig. 1b). With respect to behavioural parameters, during the 5 weeks of data collection, implanted males showed increased “beak up”, whereas control males did not (Table 2; Fig. 2b). Specifically, in implanted males the frequency of “beak up” was significantly increased in the third week after the implantation (Bonferroni post hoc test: control-week vs third week after: $P=0.025$; first week after vs third week after: $P=0.019$; Fig. 2b). In contrast, control males showed increased frequency of vigilance (Table 2; Fig. 2d)

Fig. 2 Patterns of **a,b** frequency per 5 min of “beak up”, **c,d** frequency per 5 min of vigilance and **e,f** latency of agonistic interactions in implanted (*black circles*; mean+SE) and control males (*open circles*; mean-SE) during the **a,c,e** mating season (February 1996; $n_{1im}=5$, $n_{1Co}=5$ except the control week where $n_{1Co}=4$) and the **b,d,f** early winter experiment (November 1998; $n_{2im}=7$, $N_{2Co}=7$). Data were collected 1 control week before the implantation (Co) and 4 consecutive weeks thereafter (first to fourth)



whereas implanted males did not. No further significant difference could be observed within male categories in the latency of agonistic interactions (Table 2; Fig. 2f). Also, no age-effect was found ($F_{1,69}=0.606$, $P=0.439$).

Furthermore, only during the second week after the implantation did the mean frequency of “beak up” positively and significantly correlate with TM levels in implanted, but not in control males (implanted males: $r=0.86$, $n=7$, $P=0.014$; control males: $r=-0.46$, $n=7$, $P=0.298$; Fig. 3; implanted males: control-week, $r=0.31$, $n=7$, $P=0.498$; first week after, $r=-0.54$, $n=7$, $P=0.205$; third week after, $r=0.04$, $n=7$, $P=0.939$; fourth week after, $r=-0.14$, $n=7$, $P=0.761$). As no other significant effect on behaviour was observed in implanted males (Table 2; Fig. 2d,f), the individual means were plotted over the entire period when scanning for further correlations

between TM and behaviour. No significant correlations were found (TM and vigilance: $r=0.45$, $n=7$, $P=0.306$; TM and agonistic interactions: $r=0.31$, $n=7$, $P=0.503$). Similarly in control males, individual means were plotted over the entire period in order to scan for correlations between TM and all behavioural parameters. No significant correlations were found (TM and beak up: $r=0.62$, $n=7$, $P=0.136$; TM and vigilance: $r=0.025$, $n=7$, $P=0.958$; TM and agonistic interactions: $r=-0.09$, $n=7$, $P=0.837$).

Status signalling display such as “beak up” is directed towards other members of the group. Therefore, the latency of agonistic interactions of the female partners was considered. During the second week after implantation, the female partners of implanted males were attacked faster by other flock members than the females of control males (Student’s t -test: $T=-2.49$, $n_{im,Co}=7,7$, $P=0.028$; Fig. 4;

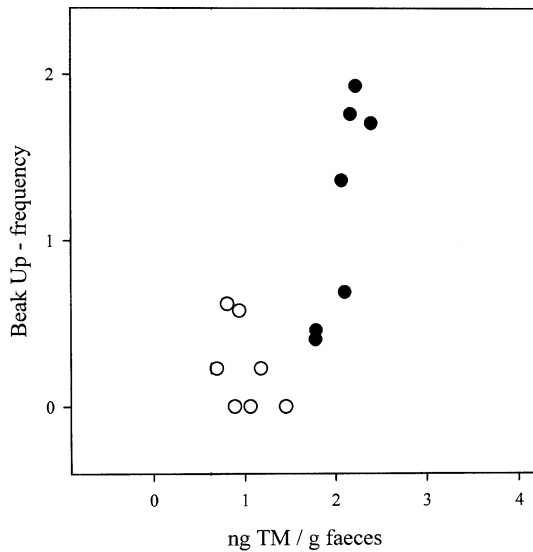


Fig. 3 In the early winter experiment, during the second week after implantation, levels of excreted testosterone immunoreactive metabolites (TM) of implanted males positively correlated with the frequency of “beak up” (implanted males: *black circles*, $r=0.86$, $n_{2im}=7$, $P=0.014$; control males: *open circles*, $r=-0.46$, $n_{2Co}=7$, $P=0.298$)

control-week, $T=-0.55$, $n_{im,Co}=7,7$, $P=0.592$; first week after, $T=0.88$, $n_{im,Co}=7,6$, $P=0.397$; third week after, $T=-0.55$, $n_{im,Co}=7,7$, $P=0.595$; fourth week after, $T=1.37$, $n_{im,Co}=7,7$, $P=0.196$).

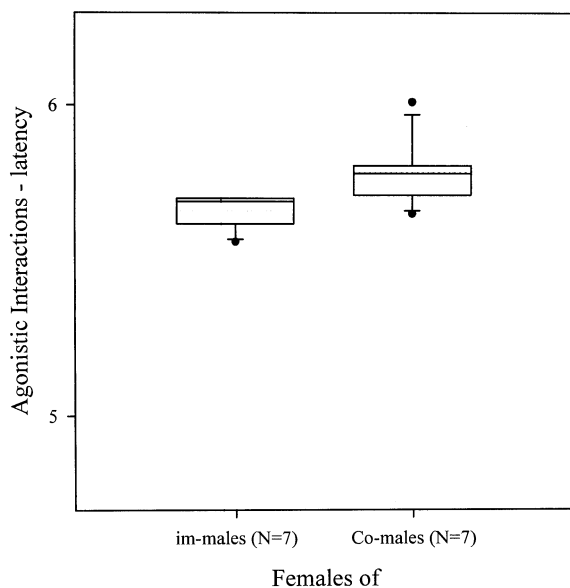


Fig. 4 In the early winter experiment, during the second week after implantation, the latency of agonistic interactions was significantly lower in the female partners of implanted males than in the female partners of control males, i.e. female partners of implanted males were faster attacked by other flock members than female partners of control males ($T=-2.492$, $n_{im,Co}=7,7$, $P=0.028$). *Full lines* median and inter-quartile ranges; *dotted lines* arithmetic mean; *whiskers* range between 10th percentile and 90th percentile; *full circles* data outside 10th percentile and 90th percentile

Comparison between seasons

As expected, marked seasonal differences were found in the levels of both steroids in both implanted and control males. Excreted levels of testosterone immunoreactive metabolites (TM) were significantly higher in the mating season as compared to the winter situation (Table 2; Fig. 1a,b). In turn, excreted levels of corticosterone immunoreactive metabolites (BM) were significantly lower in the mating season as compared to the winter situation (Table 2; Fig. 1c,d). Furthermore, in implanted males, the frequency of vigilance was significantly higher during the mating season as compared to the early winter (Table 2; Fig. 2c,d), while no other significant differences could be found between the mating and the winter season in behavioural parameters, frequency of “beak up” (Table 2; Fig. 2a,b) and latency of agonistic interactions (Table 2; Fig. 2e,f). In control males, significant differences could be found between the mating and the winter season in the frequency of “beak up” (Table 2; Fig. 2a,b) but not in the frequency of vigilance (Table 2; Fig. 2c,d) or in the latency of agonistic interactions (Table 2; Fig. 2e,f).

Discussion

Only during the non-reproductive season (November), testosterone-supplemented ganders excreted significantly more testosterone immunoreactive metabolites (TM; Fig. 1b) and showed a significantly higher frequency of the status signalling “beak up” (Fig. 2b). The missing effect of the implantation during the reproductive season (February; Figs. 1, 2) might be due to seasonal T maxima (Wingfield et al. 1990; Hirschenhauser et al. 1999a). The system was probably saturated at the receptors’ level and additional T did not produce any further effect (Hutchison 1976; Beletsky et al. 1990; Groothuis and Meeuwissen 1992). This is even more remarkable, as steroid excretion seems to be more efficient in spring than in autumn (Kotrschal et al. 2000). We assume that there may also have been more carrier protein, which could have buffered additional T and would have made circulating T inert to inactivation and excretion. Bound T proportions in the plasma may account for up to 90% of the total and may therefore constitute a circulating pool that can intensify or moderate the hormone’s action (Breuner and Orchinik 2002; see also Bentley 1998). Consistent with the known seasonal patterns in geese (Hirschenhauser et al. 1999a), levels of excreted TM decreased in control males while they showed a slight increasing trend in implanted males (Fig. 1a). It is unlikely that an insufficient size of our implant caused these negative results, because the same implant did produce significant and specific effects in early winter (Figs. 1, 2), and similarly sized implants (relative to body size) were successfully used in experiments with other bird species (e.g. Wingfield 1984; Gyger et al. 1988; Dufty 1989; Saino et al. 1995). However, our implant was quite thick and probably released little

testosterone, which could also explain the lack of an effect in February.

Most of the recent T implantation experiments were carried out during the late breeding season, at already decreasing seasonal T levels (e.g. Searcy and Wingfield 1980; Schluessner et al. 1985; Hegner and Wingfield 1987; Beletsky et al. 1990), whereas we implanted shortly after the onset of the mating period, at peaking seasonal T levels (Hirschenhauser et al. 1999a, 2000b). In fact, the time window available for us to implant was restricted to the few weeks of male sexual activity during the mating season.

Furthermore, during the mating season, both implanted and control individuals belonged to different social categories within the flock (Table 1). As T levels decrease with age (in quail; Balthazart et al. 1984), and male greylag geese from different social categories show differences in amplitude and time course of seasonal T patterns (Hirschenhauser et al. 1999a), considerable individual variation among a small sample might have affected the results (Fig. 1a). In fact, during the mating season the results were affected by the age of the focal geese. Hence, in November we focussed on males from only one social category (Table 1), avoiding a possible effect of age.

In early winter (November), T is significantly lower than during the reproductive period, although not at minimum, (Dittami 1981; Hirschenhauser et al. 1999a). Yet, our implants produced a significant increase of TM within a week (Fig. 1b; Schluessner et al. 1985; Dufty 1989; Saino et al. 1995). A significant effect on behaviour was only observed after another week (Figs. 2, 3). This is a typical time course for steroid-primed or steroid-modulated behavioural changes (Nelson 1995) and supports the hypothesis of a direct influence of T on male-type behaviour (Owens and Short 1995; Fusani et al. 1997).

The only significant effect of experimentally enhanced T was found for the “beak up” behaviour. This is a vigilance-like action pattern, with body and neck vertically stretched and the beak in an upward position, making a male quite conspicuous. “Beak up” is performed mainly by socially high-ranking individuals within the flock, i.e. paired males with offspring (e.g. Lazarus and Inglis 1978; Lamprecht 1986; Black and Owen 1986). It peaks from mating until shortly after hatching of the young (Lorenz 1988) and hence parallels the annual peak in TM (Hirschenhauser et al. 1999a). However, status signalling may be important year-round in this permanent and long-term pairbond system. As social pairing may occur year-long (Hemetsberger 2001), any challenge by a male rival would have also an indirect sexual context throughout the entire year. Hence, the status signalling “beak up” is T-sensitive. In reverse of this argument, the function of “beak up” as a status signal rather than a vigilance posture is supported by the lack of a clear effect of elevated T levels on the frequency of vigilance behaviour of implanted males (Fig. 2c,d). In this respect the decrease in vigilance frequency in February might reflect seasonal

changes in the composition of the flock just ahead of the breeding period (Lorenz 1988).

Furthermore, in early winter, the female partners of implanted males were significantly faster attacked by other flock members than the female partners of control males (Fig. 4). At the mechanistic level, this may be a kind of “redirected agonistic action” (Tinbergen 1940) caused by the mismatch between the frequency of “beak up” behaviour of the implanted males and their general status (i.e. outside the reproductive season and without offspring). It was probably less risky to attack the females, who are always slightly lower in rank than their males and may be less prone to launch a counter-attack (Lamprecht 1986; Konrad Lorenz Forschungsstelle, unpublished records).

In contrast to expectations, experimentally enhanced T did not affect aggressive behaviour in the two seasons (Fig. 2e,f). However, aggression and T are not always correlated (e.g. Pröve 1978; Tsutsui and Ishii 1981; Sapolsky 1983; Dittami and Reyer 1984). Several studies showed that T-dependent aggressive behaviour may be restricted to the reproductive season (e.g. Logan and Wingfield 1990; Schwabl and Kriner 1991; Chandler et al. 1997; Hirschenhauser et al. 2000b). According to the “challenge hypothesis”, androgens should, in fact, be involved in reproductively motivated agonistic interactions rather than in other forms of aggression (Wingfield et al. 1990). In our study, T levels were at their annual maximum during the mating season (Hirschenhauser et al. 1999a) and ganders were not responding to additional hormonal stimulation (Wingfield et al. 1990; Hirschenhauser et al. 2000b). In November, the agonistic interactions between flock members are, largely, motivated by access to resources other than mates and, therefore, were not T-sensitive.

Concluding, “beak up” as a status signal, but not aggression per se is of year-long importance for greylag ganders in a socio-sexual context. “Beak up” may be an important signal in maintaining their pairbond and their status within the flock. Hence, in the non-breeding season, T may contribute to fitness through social interactions and foraging success.

Acknowledgements M. Strobach, C. Szedlarik and B. Weiss helped with catching the geese; J. Hemetsberger helped with catching and implanting. B. Wallner, A. Schöbitz and R. Hackl assisted with the EIA in the laboratory. J. Daisley and I. Scheiber commented on an early draft of the manuscript. Special thanks to J. Hemetsberger and B. Valoti for their assistance. The project was funded by FWF Projects 10483-BIO and 12914-BIO; University of Milan (Italy) provided a grant D.F. from 1995 until December 1997. Permanent support came from the “Verein der Förderer der Konrad Lorenz Forschungsstelle” and the “Herzog von Cumberland Stiftung”. The experiment complies with the current Austrian law.

References

- Balthazart J, Turek R, Ottinger MA (1984) Altered brain metabolism of testosterone is correlated with reproductive decline in aging quail. *Horm Behav* 18:330–345

- Beletsky LD, Orians GH, Wingfield JC (1990) Effects of exogenous androgen and antiandrogen on territorial and nonterritorial red-winged blackbirds. *Ethology* 85:58–72
- Bentley PJ (1998) *Comparative vertebrate endocrinology*. Cambridge University Press, Cambridge
- Bishop CM, Hall MR (1991) Non-invasive monitoring of avian reproduction by simplified faecal steroid analysis. *J Zool* 224:649–668
- Black JM, Owen M (1986) Determinants of social rank in goose flocks: acquisition of social rank in young geese. *Behaviour* 102:129–146
- Breuner CW, Orchinik M (2002) Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J Endocrinol* 175:99–112
- Canoine V, Gwinner E (2002) Seasonal differences in the hormonal control of territorial aggression in free-living European stonechats. *Horm Behav* 41:1–8
- Carere C, Groothuis TGG, Möstl E, Daan S, Koolhaas JM (2003) Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. *Horm Behav* 43:540–548
- Cawthorn JM, Morris DL, Ketterson ED, Nolan V Jr (1998) Influence of experimentally elevated testosterone on nest defence in dark-eyed juncos. *Anim Behav* 56:617–621
- Chandler CR, Ketterson ED, Nolan V Jr (1997) Effects of testosterone on use of space by male dark-eyed juncos when their mates are fertile. *Anim Behav* 54:543–549
- Cockrem JF, Rounce JR (1994) Faecal measurements of oestradiol and testosterone allow the non-invasive estimation of plasma steroid concentration in the domestic fowl. *Br Poult Sci* 35:433–443
- Dittami J (1981) Seasonal changes in the behaviour and plasma titres of various hormones in Barheaded Geese, *Anser indicus*. *Z Tierpsychol* 55:289–324
- Dittami JP, Reyer HU (1984) A factor analysis of seasonal, behavioural, hormonal and body weight changes in adult male barheaded geese, *Anser indicus*. *Behaviour* 90:114–124
- Dittami JP, Hoi H, Sageder G (1991) Parental investment and territorial/sexual behavior in male and female reed warblers: are they mutually exclusive? *Ethology* 88:249–255
- Dufty AM Jr (1989) Testosterone and survival: a cost in aggressiveness? *Horm Behav* 23:185–193
- Fusani L, Beani L, Lupo C, Dessi-Fulgheri F (1997) Sexually selected vigilance behaviour of the grey partridge is affected by plasma androgen levels. *Anim Behav* 54:1013–1018
- Groothuis T, Meeuwissen G (1992) The influence of testosterone on the development and fixation of the form of displays in two age classes of young black-headed gulls. *Anim Behav* 43:189–208
- Gwinner E, Rödl T, Schwabl H (1994) Pair territoriality of wintering stonechats-behavior, functions and hormones. *Behav Ecol Sociobiol* 34:321–327
- Gyger M, Karakashian S, Dufty AM Jr, Marler P (1988) Alarm signals in birds: the role of testosterone. *Horm Behav* 22:305–314
- Hegner RE, Wingfield JC (1986) Behavioral and endocrine correlates of multiple brooding in the semicolonial house sparrow *Passer domesticus*. I. Males. *Horm Behav* 20:294–312
- Hegner RE, Wingfield JC (1987) Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* 104:462–469
- Hemetsberger J (2001) Die Entwicklung der Grünauer Graugansschar (*Anser anser*) seit 1973. In: Kotschal K, Müller G, Winkler H (eds) Konrad Lorenz und seine verhaltensbiologischen Konzepte aus heutiger Sicht. Filander, Fürth, pp 249–260
- Hirschenhauser K (1998) Steroidhormone aus Kot und Sozialverhalten bei Graugänsen (*Anser anser*). PhD thesis, Institute of Zoology, University of Vienna, Austria
- Hirschenhauser K, Möstl E, Kotschal K (1999a) Seasonal patterns of sex steroids determined from feces in different social categories of greylag geese (*Anser anser*). *Gen Comp Endocrinol* 114:67–79
- Hirschenhauser K, Möstl E, Kotschal K (1999b) Within-pair testosterone covariation and reproductive output in greylag geese *Anser anser*. *Ibis* 141:577–586
- Hirschenhauser K, Möstl E, Peczezy P, Wallner B, Dittami J, Kotschal K (2000a) Seasonal relationships between plasma and fecal testosterone in response to GnRH in domestic ganders. *Gen Comp Endocrinol* 118:262–272
- Hirschenhauser K, Möstl E, Wallner B, Dittami J, Kotschal K (2000b) Endocrine and behavioural response of male greylag geese (*Anser anser*) to pairbond challenges during the reproductive season. *Ethology* 106:63–77
- Houck LD, Woodley SK (1995) Field studies of steroid hormones and male reproductive behaviour in amphibians. In: Heatwole H, Sullivan B (eds) *Amphibian biology*, vol 2. Social behaviour. Surrey Beatty, Chipping Norton, UK, pp 677–703
- Hoysak DJ, Ankney CD (1996) Correlates of behavioural dominance in mallards and American black ducks. *Anim Behav* 51:409–419
- Hunt KE, Hahn TP, Wingfield JC (1997) Testosterone implants increase song but not aggression in male Lapland longspurs. *Anim Behav* 54:1177–1192
- Hutchison JB (1976) Hypothalamic mechanism of sexual behaviour, with special reference to birds. *Adv Study Behav* 6:159–200
- Ketterson ED, Nolan V Jr (1992) Hormones and life histories: an integrative approach. *Am Nat* 140:S33–S62
- Ketterson ED, Nolan V Jr, Wolf BL, Ziegenfus C (1992) Testosterone and avian life histories: effects of experimentally elevated testosterone on behavior and correlates of fitness in the dark-eyed junco (*Junco hyemalis*). *Am Nat* 140(6):980–999
- Knapp R, Moore MC (1996) Male morphs in three lizards, *Urosaurus ornatus*, have different delayed hormonal responses to aggressive encounters. *Anim Behav* 52:1045–1055
- Kotschal K, Hirschenhauser K, Möstl E (1998) The relationship between social stress and dominance is seasonal in greylag geese. *Anim Behav* 55:171–176
- Kotschal K, Dittami J, Hirschenhauser K, Möstl E, Peczezy P (2000) Effects of physiological and social challenges in different season on fecal testosterone and corticosterone in male domestic geese. *Acta Ethol* 2:115–122
- Krawany M (1996) Die Entwicklung einer nichtinvasiven Methode zum Nachweis von Steroidhormonmetaboliten im Kot von Gänsen. PhD thesis, Institute of Biochemistry Veterinary, University of Vienna, Austria
- Lamprecht J (1986) Structure and causation of the dominance hierarchy in a flock of bar-headed geese (*Anser indicus*). *Behaviour* 96:28–48
- Lazarus J (1978) Vigilance, flock size and domain of danger size in the white-fronted goose. *Wildfowl* 29:135–145
- Lazarus J, Inglis IR (1978) The breeding behaviour of the Pink-footed goose: parental care and vigilant behaviour during the fledging period. *Behaviour* 65:62–88
- Logan CA, Wingfield JC (1990) Autumnal territorial aggression is independent of plasma testosterone in mockingbirds. *Horm Behav* 24:568–581
- Lorenz K (1988) *Hier bin ich—wo bist du? Ethologie der Graugans*. Piper, Munich
- Martin P, Bateson P (1986) *Measuring behaviour*. Cambridge University Press, Cambridge
- Miller MW, Hobbs NT, Sousa MC (1991) Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis*): reliability of cortisol concentrations in urine and feces. *Can J Zool* 69:15–24
- Moore MC, Thompson CW (1990) Field endocrinology in reptiles: hormonal control of alternative male reproductive tactics. In: Epple A, Scanes CG, Stetson MH (eds) *Progress in comparative endocrinology*. Wiley/Liss, New York, pp 685–690
- Möstl E, Meyer HH, Bamberg E, von Hegel G (1987) Oestrogen determination in faeces of mares by EIA on microtiter plates. *Proceedings of the Symposium on the Analysis of Steroids*, Sopron, Hungary, pp 219–224

- Nelson RJ (1995) An introduction to behavioural endocrinology. Sinauer, Sunderland, Mass.
- Oliveira RF, Almada VC, Canario AVM (1996) Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. *Horm Behav* 30:2–12
- Owens IPF, Short RV (1995) Hormonal basis of sexual dimorphisms in birds: implications for new theories of sexual selection. *Tree* 10:44–47
- Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused 14C-steroid hormones via feces and urine in domestic livestock. *Anim Reprod Sci* 43:43–63
- Pfeifer A (1991) Datenanalyse mit SPSS/PC+4.0. Oldenbourg, Munich
- Pröve E (1978) Quantitative Untersuchungen zu Wechselbeziehungen zwischen Balzaktivität und Testosterontitern bei männlichen Zebrafinken (*Taeniopygia guttata castanotis* Gould). *Z Tierpsychol* 48:47–67
- Raveling DG (1970) Dominance relationships and agonistic behaviour of Canada geese in winter. *Behaviour* 37:291–319
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43:223–225
- Rich EL, Romero ML (2001) Daily and photoperiod variations of basal and stress-induced corticosterone concentration in house sparrows (*Passer domesticus*). *J Comp Physiol B* 171:543–547
- Rissman E, Wingfield JC (1984) Hormonal correlates of polyandry in the spotted sandpiper, *Actitis macularia*. *Gen Comp Endocrinol* 56:401–405
- Ros AFH (1999) Effects of testosterone on growth, plumage pigmentation, and mortality in black-headed gull chicks. *Ibis* 141:451–459
- Saino N, Møller AP (1995) Testosterone-induced depression of male parental behavior in the barn swallow: female compensation and effects on seasonal fitness. *Behav Ecol Sociobiol* 36:151–157
- Saino N, Møller AP, Bolzern AM (1995) Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav Ecol* 6:397–404
- Sapolsky RM (1983) Endocrine aspects of social instability in the olive baboon (*Papio anubis*). *Am J Primatol* 5:365–379
- Schleussner G, Dittami JP, Gwinner E (1985) Testosterone implants affect moult in male European starlings *Sturnus vulgaris*. *Physiol Zool* 58:597–604
- Schoech SJ, Ketterson ED, Nolan V Jr (1999) Exogenous testosterone and the adrenocortical response in dark-eyed juncos. *Auk* 116:64–72
- Schütz K, Wallner B, Kotrschal K (1997) Diurnal patterns of steroid hormones from feces in greylag goslings (*Anser anser*). In: Taborsky M, Taborsky B (eds) *Advances in Ethology* 32, p 105
- Schwabl H (1992) Winter and breeding territorial behaviour and levels of reproductive hormones of migratory European robins. *Ornis Scand* 23:271–276
- Schwabl H, Kriner E (1991) Territorial aggression and song of male European robins (*Erithacus rubecula*) in autumn and spring: effects of antiandrogen treatment. *Horm Behav* 25:180–194
- Searcy WA, Wingfield JC (1980) The effects of androgen and antiandrogen on dominance and aggressiveness in male red-winged blackbirds. *Horm Behav* 14:126–135
- Silverin B (1980) Effects of long-acting testosterone treatment on free-living pied flycatchers, *Ficedula hypoleuca*, during the breeding period. *Anim Behav* 28:906–912
- Sorenson LG, Nolan PM, Brown AM, Derrickson SR, Monfort SL (1997) Hormonal dynamics during mate choice in the northern pintail: a test for the “challenge hypothesis”. *Anim Behav* 54:1117–1133
- Tinbergen N (1940) Die Übersprungbewegung. *Z Tierpsychol* 4:1–40
- Tsutsui K, Ishii S (1981) Effects of sex steroids on aggressive behavior of adult male Japanese quail. *Gen Comp Endocrinol* 44:480–486
- Waldenberger F, Kotrschal K (1993) Individual vigilance in male greylag geese (*Anser anser*) depends on flock density and social status. *Oekol Voegel* 15:193–199
- Wikelski M, Hau M, Wingfield JC (1999) Social instability increases plasma testosterone in a year-round territorial neotropical bird. *Proc R Soc Lond B* 266:551–556
- Wingfield JC (1984) Androgens and mating systems: testosterone-induced polygyny in normally monogamous birds. *Auk* 101:665–671
- Wingfield JC, Hahn TP (1994) Testosterone and territorial behaviour in sedentary and migratory sparrows. *Anim Behav* 47:77–89
- Wingfield JC, Ramenofsky M (1985) Testosterone and aggressive behaviour during the reproductive cycle of male birds. In: Gilles R, Balthazart J (eds) *Neurobiology*. Springer, Berlin Heidelberg New York, pp 92–104
- Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF (1990) The “challenge hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. *Am Nat* 136:829–846
- Wingfield JC, Jacobs J, Hillgarth N (1999) Ecological constraints and the evolution of hormone-behaviour interrelationships. *Am Nat* 136:829–846
- Woodroffe R, Macdonald DW, Cheeseman CL (1997) Endocrine correlates of contrasting male mating strategies in the European badger (*Meles meles*). *J Zool* 241:291–300
- Zöfel P (1992) *Statistik in der Praxis*, 3rd edn. UTB, Stuttgart