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Seasonal Patterns of Sex Steroids Determined from Feces in Different Social Categories of Greylag Geese (*Anser anser*)

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Seasonal patterns of fecal 17β-OH-androgen, estrogen, and progesterone equivalents of male and female greylag geese (*Anser anser*) were analyzed in a flock of free-living geese. These were compared among social categories determined by pairbond status and breeding success. The annual cycle was divided into 13 phases. Phasewise intra-sexual comparisons were made between social categories. The seasonal variation obtained from feces was in general agreement with the literature on plasma patterns in geese and other temperate-zone birds. However, there were distinct differences in seasonal hormone patterns among the social categories. In unpaired males, androgen was elevated for a longer period of time during sexually active phases compared with paired males. In male geese, high levels of androgen did not interfere with parenting but were related to pairbond status, whereas in females, androgen and progesterone were positively related to parental behavior. In the Fall, androgen, progesterone, and estrogen peaked only in unpaired males. In unsuccessful females, estrogen started to increase earlier in the Winter and was higher in amplitude and duration than that in females guarding offspring. In general, fecal steroids showed a clear-cut difference only between sexually active and parental phases of the year in the successfully breeding pairs, whereas unpaired males retained a hormonal state closer to sexually active phases throughout the year.

Key Words: Anser anser; feces; noninvasive endocrine monitoring; 17β-OH-androgen; estrogen; progesterone; males; females; pairing status; parental investment; seasonality; Fall sexuality.

The annual cycle of greylag geese (*Anser anser*) consists of Spring migration, breeding, molt, Fall migration, and the reaggregation into a Winter flock in early Fall (Lorenz, 1979; Rutschke, 1982). Each of these phases is characterized by characteristic behavioral and hormonal changes (Akesson and Raveling, 1981, 1984; Dittami, 1981; Farner and Wingfield, 1980; Temple, 1974; Wingfield and Farner, 1980).

The present data are based on noninvasive sampling and the determination of steroid equivalents in feces. Fecal sampling avoids capture and handling stress and therefore enables the recording of seasonal patterns of excreted steroid equivalents without interference. Only noninvasive sampling permits collection of large numbers of samples from unrestrained animals. Methodological developments have allowed the analysis of gonadal steroids as well as corticosterone in avian feces (Hirschenhauser *et al.*, 1999; Kotrschal *et al.*, 1998; Krawany, 1996; Lee *et al.*, 1995; Wasser *et al.*, 1997). In mammals and birds, fecal steroid equivalents seem to parallel plasma concentrations (Bishop and Hall, 1991;

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Cockrem and Rounce, 1994; Peter *et al.,* 1996; Wasser *et al.*, 1993; Wasser *et al.,* 1997). Fecal steroid measurements may, therefore, reliably reflect plasma levels.

Previous work indicated amplitudinal and temporal differences in the seasonal patterns of androgen, estrogen, and progesterone due to the individuals' involvement in nesting and / or parenting (Akesson and Raveling, 1981, 1984; Dittami, 1981; Hannon and Wingfield, 1990; Hirschenhauser *et al.,* 1997). Physiologically, androgen plays a role in reproduction in both sexes. In males it regulates sperm production and the activation of sexual behavior (Podestá and Rivarola, 1974; Ramenofsky, 1984; Temple, 1974). Female androgen levels are maximal during the pre-laying phase and correspond with follicular development, receptivity, and sexual behavior (Carter, 1992; Furr and Thomas, 1970; Johnson, 1986; Schwabl, 1996). Decreased androgen in both sexes during the end of incubation, molt, and Summer photorefractoriness was anticipated, since in altricial species high androgen levels suppress male parental care (Dittami *et al.,* 1991; Farner and Wingfield, 1980; Hegner and Wingfield, 1987; Saino and Moller, 1995; Schoech *et al.,* 1998; Silverin, 1990) and molt (Péczely, 1992).

In female waterfowl estrogen is high when ovaries contain mature follicles and declines during incubation (Bluhm *et al.,* 1983; Dawson, 1983; Donham, 1979; Harvey *et al.,* 1981). Estrogen is involved in yolk protein synthesis, female reproductive behavior, and nest building (Akesson and Raveling, 1984; Bluhm *et al.,* 1983; Farner and Wingfield, 1980). Progesterone in female birds is produced by the thecal cells of mature ovarian follicles and by the adrenals (Bolaños *et al.,* 1997; Budec *et al.,* 1996). Progesterone may induce ovulation (Johnson and van Tienhoven, 1980) and maximal concentrations were associated with egg laying in female birds (Bluhm *et al.,* 1983; Dawson, 1983; Dick *et al.,* 1978; Donham, 1979; Harvey, *et al.,* 1981; Johnson, 1986). This corresponds with the hypothesis that progesterone has its behavioral role later than estrogen and coincides with the initiation of incubation in the presence of estrogen (Johnson and van Tienhoven, 1980; Sharp and Lea, 1996). Also, progesterone may affect the onset of molt in the domestic goose (*Anser domesticus;* Péczely, 1992) and it is an obligate precursor for androgen and estrogen (Nelson, 1995). High levels of progesterone outside the breeding season were suggested to be due to extra-gonadal production in both sexes (Davis *et al.,* 1995; Dawson, 1983; McCreery and Farner, 1979). However, this does not explain why testes and adrenal glands of adult male birds (Farner and Wingfield, 1980) produce (and they also excrete) relatively high amounts of progesterone throughout the year (Bezzel and Prinzinger, 1990; Fowler *et al.,* 1994; Johnson, 1986; Withers, 1992). In avian plasma (Donham, 1979; Harvey *et al.,* 1981; Johnson and van Tienhoven, 1980) sex-specific differences have been suggested to reflect the circannual timing of progesterone fluctuations rather the amplitudes. Comparatively little is known about the function of progesterone in reproductive and / or parental behavior of male birds (Davis *et al.,* 1995; Dawson, 1983; McCreery and Farner, 1979; Wingfield and Farner, 1978a). The aim of this study was to generate functional hypotheses by examining steroid profiles in a social perspective.

The present study focusses on the gonadal and adrenal steroid hormones. Individual fecal samples were collected throughout a complete annual cycle across all social categories within the flock. The purpose was to describe seasonal changes in levels of excreted equivalents of 17β-OH-androgen, estrogen, and progesterone. Differences among social categories as defined by pairbond status and breeding success were sought and it is proposed that there are different physiological requirements that depend on individual pairbond status or the presence of offspring. For example, the presence or absence of a long-term partner during the mating phases may influence the individual timing and duration of steroid increases (Kotrschal *et al.,* 1998; Wingfield *et al.,* 1990). Therefore, results are discussed in the light of the individual social environment. Differences between reproductively successful and unsuccessful individuals, the transition from sexual to parental behavior (Farner and Wingfield, 1980; Temple, 1974), and the post-refractory peak of sexual activity in Fall (Campbell *et al.,* 1978; Dittami, 1981; Péczely *et al.,* 1993) were also assessed.

METHODS

Animals and Data Collection

A free-living and nonmigratory flock of greylag geese was introduced into the Upper Austrian valley

of the river Alm in 1973 (Lorenz, 1979). The geese are all-year residents and are provided with supplemental food year round. Birds are individually marked with colored leg bands and are habituated to the presence of humans. This allows sampling of individual feces (by visual observation) year round even during breeding, hatching, and molt. Individual life history parameters, such as social interactions and breeding performance, have been recorded since 1973. This study includes 42 males and 31 females. Individual fecal samples were collected weekly from February 1993 to February 1994, between 08.00 AM and 11.00 AM. A total of 1574 fecal samples, 940 from males and 634 from females, were frozen within 2 h after collection and stored at −80°C until assayed. On average 21.7 ± 0.8 fecal samples were collected per individual per year.

Data were analyzed for differences among social categories (paired versus unpaired individuals, successful versus unsuccessful breeders). *Family males* ($n = 14$) and *family females* ($n = 14$) are individuals in the company of their long-term mates and their offspring of the same year. *Paired males* ($n = 18$) and *paired females* ($n = 17$) are paired individuals without offspring. *Singletons* ($n = 10$) are adult unpaired males, mainly widowers which had lost their mates to predators during incubation. There were no unpaired adult females available in the year sampled.

Measurement of Steroid Hormones from Feces

To date, studies on fecal steroid excretion in birds such as for sex determination (Bercovitz *et al.*, 1978) and reproductive activity (Bercovitz *et al.*, 1982, 1983; Bishop and Hall, 1991) have employed radioimmunoassays without prior hydrolysis or enzymatic deconjugation of the samples. HPLC separation of metabolites revealed that, in contrast to steroids from chicken feces (Kikuchi *et al.*, 1994; Wallpach, 1998), geese excreted steroids predominantly in conjugated forms via feces and urine, respectively (Krawany, 1996). Therefore, β-glucoronidase-arylsulfatase was used for deconjugation.

Fecal steroid equivalents were then determined by enzyme immunoassay (Möstl *et al.*, 1987) using group-specific antibodies against 4-androstene-17β-ol-3-on-carboxymethyloxine-albumine-CMO (rabbit), oestradiol-17β-17-hemisuccinate-albumine (sheep), and progesterone-3α-ol-hemisuccinate (rabbit). As labels 5 α-androstane-3β, 17β-diol-3-hemisuccinate, oestradiol-17β-17-glucuronide, and progesterone-3-CMO (Palme and Möstl, 1993) were used for biotinylation (dioxaoctane-biotin). Assay concentration limits for reliable measurement ranged from 0.4 to 30 pg/well for androgen, from 0.3 to 50 pg/well for estrogen, and from 2.1 to 500 pg/well for progesterone. The specificity of the estrogen and progesterone test systems used, determined as described by Palme *et al.* (1997) are shown in Table 1; specificity for the androgen assay has been described (Hirschenhauser *et al.*, 1999).

Fecal samples (0.5 g) were extracted with 1 ml water plus 1.5 ml methanol by vortexing (30 min). Thereafter, an aliquot of each sample was evaporated to dryness,

TABLE 1a
Specificity of the Estrogen Assay According to Standard Test Procedures from the Lab of E. Möstl

Crossreactivity with	(%)
1,3,5(10)-Estratrien-3,16α,17β-triol (Oestriol)	141.0
1,3,5(10)-Estratrien-3-ol-17β-diol (Oestradiol-17β)	82.0
1,3,5(10),7-Estratetraen-3,17β-diol	77.3
1,3,5(10)-Estratrien-3-ol-17-one (Oestrone)	68.0
1,3,5(10),7-Estratetraen-3-ol-17-one	24.2
1,3,5(10)-Estratrien-3-ol-17α-diol (Oestradiol-17α)	21.0
1,3,5(10),6,8-Estrapentaen-3,17β-diol	1.1
1,3,5(10),6,8-Estrapentaen-3-ol-17-one	0.8
1,3,5(10)-Estratrien-3-ol-17-on-3-sulfate	<0.0
1,3,5(10)-Estratrien-3-ol-17-on-3-glucuronide	<0.0

TABLE 1b
Specificity of the Progesterone Assay According to Standard Test Procedures from the Lab of E. Möstl

Crossreactivity with	(%)
Progesterone	100.0
5α-Pregnane-3,20-dione	18.2
5β-Pregnane-3,20-dione	18.2
Pregnenolone	16.7
5α-Pregnane-3β-ol-20-one	16.0
4-Pregnen-11β-ol-3,20-dione	6.5
11-Desoxycorticosterone	3.5
4-Pregnen-17α-ol,3-one	1.9
5β-Pregnane-3β-ol-20-one	1.4
5β-Pregnane-3α-ol-20-one	1.3
4-Pregnen-20α-ol-3-one	<1.0
4-Pregnen-20β-ol-3-one	<1.0
4-Pregnen-17α,20α-diol,3-one	<1.0
5β-Pregnane-3α,20α-diol	<0.0
Cortisol	<0.0

redissolved in 500 µl acetate buffer (pH 4.8), and hydrolyzed using 500 µl of a 1:500 diluted mixture of β-glucuronidase / arylsulfatase (Merck 4114). The hydrolysis of methanol-extracted samples led to 36.0 ± 14.5% of unconjugated substances for androgen and 67.3 ± 28.1% for estrogen. The effectivity of hydrolysis was not examined for progesterone for organizational reasons. For determination of intra- and inter-assay variations homogenized pooled samples were used. Mean intra-assay coefficient of variation for the fecal samples was 9.5% for androgen, 6.9% for estrogen, and 9.6% for progesterone. Mean inter-assay coefficient of variation was 19.6% for androgen, 14.0% for estrogen, and 18.8% for progesterone.

Data Processing and Statistics

The annual cycle was divided into 13 biologically significant phases. In the studied flock, the onset of egg laying was spread over 1 month. Therefore, it was necessary to standardize the individual data according to the timing of reproductive events to make them cohesive (Dittami, 1981). Definitions of the phases are listed in Table 2. The phases in nonnesting singletons were fitted accordingly, as defined by the average number of days that had determined the phases of (successfully) breeding individuals (Table 2). Most paired females (even though without breeding success) attempted to breed; hence, phases were determined by the date of laying their first egg and in females that did, by the initiation of incubation. As soon as these females ceased nesting, phase durations were determined as in the singletons.

Wherever possible, mean steroid values ± SE per phase per individual were calculated. During the incubation phases (*ph*5 + *ph*6) samples from only a few females were available.

All comparisons were done within and between phases (*ph*1–*ph*13). A parametric multivariate ANOVA was used with all hormone equivalents to distinguish the effects of season and social category. Nonparametric analyses of variance for dependent variables were used in within-category comparisons (Friedman and Wilcoxon test). Comparisons between social categories were tested as independent variables (Mann Whitney *U* test). All probabilities are given as two-tailed; Bonferroni post hoc corrections were applied.

TABLE 2
The Phases for the Standardization of Individual Data According to the Timing of Reproductive Events

	Reproductive season	
*ph*1	Phase 1	Courtship in February
*ph*2	Phase 2	Mating I, 4 to 2 weeks before the first egg was laid
*ph*3	Phase 3	Mating II, the 2 weeks before the first egg was laid
*ph*4	Phase 4	Egg laying
*ph*5	Phase 5	Incubation I, first 2 weeks of incubation
*ph*6	Phase 6	Incubation II, last 2 weeks of incubation
	Parental season	
*ph*7	Phase 7	Hatching, 2 weeks after the female had left the nest
*ph*8	Phase 8	Molt, flightless period in June / July
*ph*9	Phase 9	Summer flock in August / September
*ph*10	Phase 10	'Pre-migratory' flock in October
*ph*11	Phase 11	November, peak 'pre-migratory' fattening
*ph*12	Phase 12	Winter flock in December
*ph*13	Phase 13	Pre-courtship in January

Note. Individual phases were time-shifted according to laying the first egg. (*ph* number: abbreviations as in the text).

RESULTS

17β-OH-Androgens

Male social categories. ANOVA demonstrated a significant effect of phase and social category ($F = 3.99$, $df = 24$, $P = 0.000$; Fig. 1A). Family males and paired ganders both had annually maximal androgen values 2 weeks before the first egg was laid (*ph*3) by their mated female. The family males' androgen gradually fell to low levels during molt (*ph*3 vs *ph*8: $Z = -2.20$, $n = 6$, $P = 0.055$). In contrast, androgen of the paired males decreased significantly in the laying phase (*ph*3 vs *ph*4; $Z = -2.40$, $n = 10$, $P = 0.033$). Androgen equivalents during molt (*ph*8) were at their annual minima in all gander categories. In both paired male categories levels remained low during Fall and early Winter, gradually increased in December, and reached the annual maximum at the peak of the following mating season (Fig. 1A).

In the singletons androgen remained elevated throughout all phases of the sexually active season (Friedman *ph*1–*ph*7: $\chi^2 = 4.07$, $df = 6$, $P = 0.667$). The singletons' androgen was already increased in phase 2

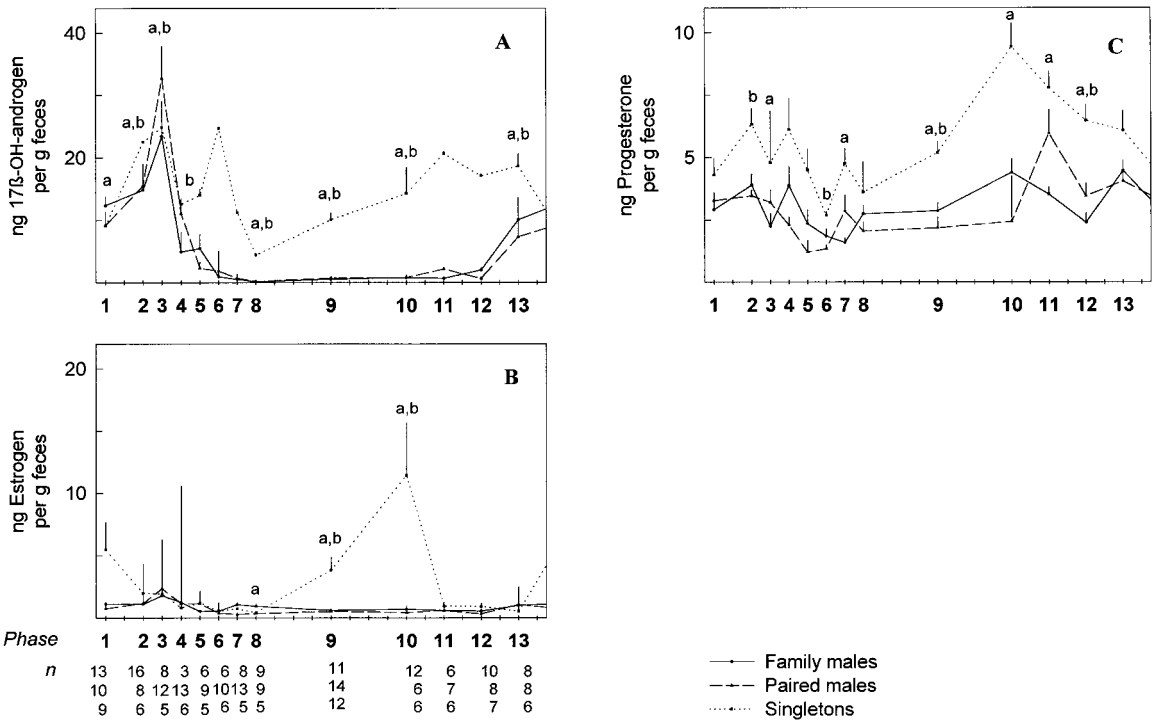

FIG. 1. Seasonal patterns of fecal 17β-OH-androgen (A), estrogen (B), and progesterone (C) from feces of the male social categories among the sampled flock of greylag geese. Phases along *x*-axis: 1, Courtship in February; 2, Mating I, 4 to 2 weeks before the first egg; 3, Mating II, the 2 weeks before the first egg; 4, Egg laying; 5, Incubation I, the first 2 weeks of incubation; 6, Incubation II, the last 2 weeks of incubation; 7, Hatching, the 2 weeks after the female had left the nest; 8, Molt, flightless period in June/July; 9, Summer flock in August/September; 10, 'Pre-migratory' flock in October; 11, November, peak 'pre-migratory' fattening; 12, Winter flock in December; 13, Pre-courtship in January. Plotted are mean values per phase (±SE); full lines, family males; hatched lines, paired males without offspring; dotted lines, unpaired adult males. Letters represent significant differences ($P < 0.05$) between (a) family and singleton males and between (b) paired and singleton males. (A) Significant differences between mean 17β-OH-androgen of family and singleton males (Mann Whitney *U* test) were found in *ph*1 ($Z = -2.30$, $P = 0.042$), *ph*2 ($Z = -3.42$, $P = 0.001$), *ph*3 ($Z = -2.64$, $P = 0.017$), *ph*8 ($Z = -2.74$, $P = 0.012$), *ph*9 ($Z = -3.63$, $P = 0.001$), *ph*10 ($Z = -3.37$, $P = 0.001$), and in *ph*13 ($Z = -2.45$, $P = 0.028$), and significant differences between mean 17β-OH-androgen of paired and singleton males were found in *ph*2 ($Z = -3.10$, $P = 0.004$), *ph*3 ($Z = -2.21$, $P = 054$), *ph*4 ($Z = -3.42$, $P = 0.001$), *ph*8 ($Z = -2.39$, $P = 0.033$), *ph*9 ($Z = -3.86$, $P = 0.000$), *ph*10 ($Z = -2.88$, $P = 0.008$), and in *ph*13 ($Z = -2.32$, $P = 0.040$). (B) Significant differences between mean estrogen of family and singleton males were found in *ph*8 ($Z = -2.33$, $P = 0.029$), *ph*9 ($Z = -3.69$, $P = 0.000$), and in *ph*10 ($Z = -3.37$, $P = 0.001$), and significant differences between mean estrogen of paired and singleton males were found in *ph*9 ($Z = -4.07$, $P = 0.000$) and in *ph*10 ($Z = -2.88$, $P = 0.008$). (C) Significant differences between mean progesterone of family and singleton males were found in *ph*3 ($Z = -2.34$, $P = 0.038$), *ph*7 ($Z = -2.93$, $P = 0.007$), *ph*9 ($Z = -3.20$, $P = 0.003$), *ph*10 ($Z = -2.90$, $P = 0.007$), *ph*11 ($Z = -2.88$, $P = 0.008$), and in *ph*12 ($Z = -3.42$, $P = 0.001$), and significant differences between mean progesterone of paired and singleton males were found in *ph*2 ($Z = -2.32$, $P = 0.040$), *ph*6 ($Z = -2.82$, $P = 010$), *ph*9 ($Z = -3.29$, $P = 0.002$), and in *ph*12 ($Z = -2.78$, $P = 0.011$).

and remained elevated for a longer period compared with both paired male categories that exhibited short-term peaks only during mating (*ph*3, Fig. 1A). Androgen equivalents were maximal at the end of April and beginning of May (*ph*6) when all of the paired males' females were already incubating. Compared with the low levels during molt (*ph*2 vs *ph*8: $Z = -2.74$, $n = 6/5$, $P = 0.012$), androgen was more than three times higher in October (*ph*8 vs *ph*10: $Z = -2.74$, $n = 6/5$, $P = 0.012$) and remained high throughout the rest of the year (Friedman *ph*9–*ph*1: $\chi^2 = 4.43$, $df = 5$, $P = 0.490$). Also during molt, Summer, and until October (*ph*8–*ph*10) the singletons significantly exceeded the other males in androgen levels (Fig. 1A).

Female social categories. ANOVA revealed a significant univariate effect of phase on androgen in females ($F = 14.63$, $df = 12$, $P = 0.000$; Fig. 2A). Social category ($F = 0.01$, $df = 1$, $P = 0.941$) and the interac-

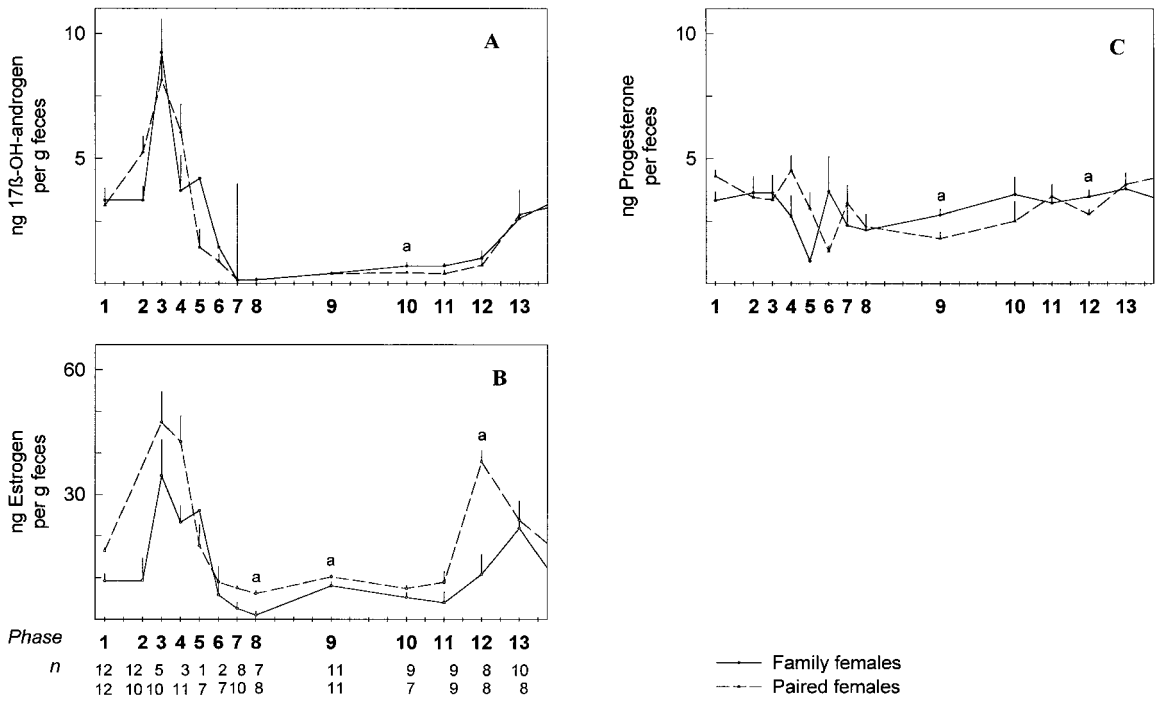

FIG. 2. Seasonal patterns of fecal 17β-OH-androgen (A), estrogen (B), and progesterone (C) from feces of the female social categories among the sampled flock of greylag geese. For definitions of the phases along *x*-axis see Fig. 1. Plotted are mean values per phase (±SE). Letters (a) represent significant differences ($P < 0.05$) between family (full lines) and paired females without offspring (hatched lines). Due to low sample sizes from family females during the incubation phases testing was omitted in *ph*5 and *ph*6. (A) The difference between mean 17β-OH-androgen of family and paired females (Mann Whitney *U* test) in *ph*10 was significant ($Z = -2.60$, $P = 0.009$). (B) Significant differences between mean estrogen of family and paired females were found in *ph*8 ($Z = -1.97$, $P = 0.049$), *ph*9 ($Z = -2.50$, $P = 0.013$), and in *ph*12 ($Z = -2.21$, $P = 0.027$). No estrogen values from paired females without offspring during *ph*2 were due to a failure in the EIA procedure. (C) Significant differences between mean progesterone of family and paired females were found in *ph*9 ($Z = -1.97$, $P = 0.049$) and in *ph*12 ($Z = -2.26$, $P = 0.024$).

tion between category and phase ($F = 1.29$, $df = 11$, $P = 0.227$) had no significant effect. The seasonal patterns of androgen were similar in the reproductively successful family females and in the paired females without offspring. All females had annual maxima during the 2 weeks before laying their first egg which coincides with the peak of mating (*ph*3, Fig. 2A). Thereafter, levels decreased to the annual minimum during molt (family females *ph*3 vs *ph*8: $Z = -2.84$, $n = 5/7$, $P = 0.009$, Bonferroni corrected; paired females *ph*3 vs *ph*8: $Z = -2.20$, $n = 6$, $P = 0.055$, Bonferroni corrected). Throughout Summer and Fall phases, androgen levels remained low and increased again in January (family females *ph*12 vs *ph*13: $Z = -2.3805$, $n = 8$, $P = 0.017$; paired females *ph*8 vs *ph*13: $Z = -2.20$, $n = 6$, $P = 0.055$, Bonferroni corrected). Although ANOVA did not indicate an effect of category by phase, the family females' androgen significantly exceeded that of the paired females in October (*ph*10, Fig. 2A). Furthermore, mean androgen in all phases of the parental season (Table 2) in the family females was significantly higher than mean 'parental' androgen of the paired females without offspring ($Z = -2.11$, $n = 14/14$, $P = 0.035$; Fig. 3).

Estrogen

Male social categories. ANOVA revealed significant effects of phase ($F = 6.86$, $df = 12$, $P = 0.000$), social category ($F = 4.82$, $df = 2$, $P = 0.008$), and the interaction between the two ($F = 8.74$, $df = 24$, $P = 0.000$) on estrogen in male geese (Fig. 1B). Family and paired males both had maximal values during the peak of the mating season (*ph*3). The family males' seasonal estrogen fluctuations were insignificant (Friedman *ph*8–*ph*3: $\chi^2 = 8.80$, $df = 8$, $P = 0.360$). In contrast,

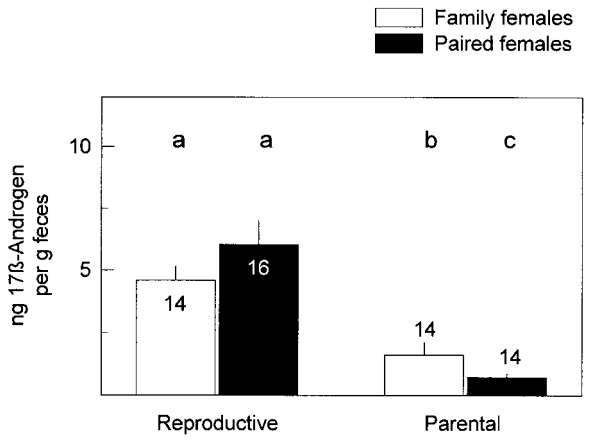

FIG. 3. Mean ± SE fecal 17β-OH-androgen per reproductive (*ph*1 to *ph*6) and per parental season (*ph*7 to *ph*13) in family females (open bars) and in paired females without offspring (black bars). During the parental season family females had higher androgen than paired females. Numbers indicate *n* individuals per season; different letters (a, b, c) represent significant differences ($P < 0.05$) between social categories and between seasons.

the paired males' levels dropped significantly during the laying phase (*ph*3 vs *ph*4: $Z = -2.70$, $n = 10$, $P = 0.014$, Bonferroni corrected). When the paired males' partners had given up on incubation and molt had started (*ph*7 + 8), estrogen excretion further decreased towards the annual minimum (*ph*4 vs *ph*7: $Z = -2.38$, $n = 8$, $P = 0.035$, Bonferroni corrected) and remained at low levels throughout the rest of the year (Friedman *ph*7–*ph*2: $\chi^2 = 10.04$, $df = 8$, $P = 0.186$).

Singletons showed a different pattern. The unpaired males had a short but significant peak in October (*ph*8 vs *ph*10: $Z = -2.74$, $n = 5/6$, $P = 0.012$, Bonferroni corrected; Fig. 1B). Except for October, estrogen in the singletons remained at low baseline levels throughout the rest of the year (Friedman *ph*11–*ph*9: $\chi^2 = 12.09$, $df = 10$, $P = 0.279$). Therefore, the increase in February (*ph*1, Fig. 1B) was not significantly different from estrogen levels in the other phases and did not differ from estrogen in the other male categories. Throughout Summer until the end of October, the singletons excreted higher amounts of estrogen equivalents than both of the other male categories (Fig. 1B).

Female social categories. Estrogen in female geese was significantly affected by phase ($F = 26.43$, $df = 12$, $P = 0.000$) and, in contrast to all other female steroids, by social category ($F = 41.71$, $df = 1$, $P = 0.000$) and the interaction between category and phase ($F = 2.78$, $df = 11$, $P = 0.002$; Fig. 2B). Both female categories had maximal estrogen in the 2 weeks before laying (*ph*3), had significant minima during hatching and molt, and showed elevated levels during the Summer period. The peak in successfully breeding family females was short-term (*ph*3) and decreased significantly to low levels during hatching and molt (*ph*3 vs *ph*7: $Z = -2.45$, $n = 3/5$, $P = 0.014$). Beginning with the elevated levels during Summer (*ph*8 vs *ph*9: $Z = -2.37$, $n = 7$, $P = 0.036$, Bonferroni corrected), the family females' estrogen was further increased in January (*ph*9 vs *ph*13: $Z = -2.37$, $n = 7$, $P = 0.036$, Bonferroni corrected; Fig. 2B).

In contrast to the family females, the paired females' pre-laying estrogen levels remained elevated throughout the laying phase (*ph*3–4) and decreased only slowly towards the annual minimum during hatching and molt (*ph*3 vs *ph*7: $Z = -2.02$, $n = 5$, $P = 0.086$, Bonferroni corrected). Beginning with the elevated Summer levels (*ph*8 vs *ph*9: $Z = -2.37$, $n = 7$, $P = 0.036$, Bonferroni corrected) the paired females' estrogen significantly dropped to low levels in October (*ph*9 vs *ph*10: $Z = -2.37$, $n = 7$, $P = 0.036$, Bonferroni corrected) and fluctuations throughout the Winter phases remained nonsignificant (Friedman *ph*10–*ph*1: $\chi^2 = 7.60$, $df = 4$, $P = 0.107$). However, during molt, Summer and in December, estrogen in paired females without offspring was significantly higher than in family females (Fig. 2B).

Progesterone

Male social categories. There were univariate effects of phase ($F = 6.46$, $df = 12$, $P = 0.000$) and of social category ($F = 45.24$, $df = 2$, $P = 0.000$) on progesterone in male geese, while the combined effect of category by phase on progesterone in male geese was not significant ($F = 1.03$, $df = 24$, $P = 0.430$; Fig. 1C).

Family males showed only marginally significant seasonal fluctuations. However, there was a marked decrease in the hatching phase (*ph*7): beginning with elevated levels throughout the Winter phases (Friedman *ph*10–*ph*2: $\chi^2 = 6.57$, $df = 5$, $P = 0.255$) progesterone reached its annual minimum during the first 2 weeks after hatching of their young (*ph*2 vs *ph*7: $Z = -2.2014$, $n = 6$, $P = 0.055$, Bonferroni corrected). Thereafter, progesterone increased slowly toward October (*ph*7 vs *ph*10: $Z = -2.20$, $n = 6$, $P = 0.055$, Bonferroni corrected) and remained elevated until the next mating phase.

In paired males without breeding success the minimum was at the beginning of the incubation phase (*ph*5). This was 1 month earlier than the family males' annual minimum. The only significant increase in the paired males' seasonal pattern was in November (*ph*5 vs *ph*11: $Z = -2.54$, $n = 8/7$, $P = 0.022$, Bonferroni corrected).

The singletons' seasonal progesterone fluctuations were only marginally significant. The minimum was at the end of April and beginning of May (*ph*6: 2.68 ± 0.16 ng progesterone / g feces) and peaks occurred in October (*ph*10: 9.44 ± 0.94 ng progesterone / g feces; Fig. 2C); these differences were nonsignificant (*ph*6 vs *ph*10: $Z = -2.02$, $n = 5$, $P = 0.086$, Bonferroni corrected). Still, throughout the year the singletons' progesterone was higher than that of both of the other (paired) male categories (Fig. 1C).

Comparison of mean progesterone of all phases of the reproductive season with mean progesterone of all phases of the parental season (Table 2) revealed a clear pattern: in the family males there was no difference between seasons ($Z = -0.50$, $n = 14$, $P = 0.616$), whereas paired and singleton males had significantly higher mean progesterone during the parental season compared with the reproductive season ($Z = -0.50$, $n = 14$, $P = 0.616$), whereas paired and singleton males had significantly higher mean progesterone during the parental season compared with the reproductive season ($Z = -2.22$, $n = 16$, $P = 0.026$; $Z = -2.38$, $n = 8$, $P = 0.017$; Fig. 4). During sexually active phases of the year mean progesterone was highest in unpaired males and lowest in paired males without offspring ($Z = -3.72$, $n = 10/18$, $P = 0.0002$; Fig. 4).

Female social categories. The univariate effect of phase on progesterone in female geese was significant ($F = 2.51$, $df = 12$, $P = 0.003$), but not social category ($F = 1.10$, $df = 1$, $P = 0.294$) or the interaction between the two ($F = 1.43$, $df = 11$, $P = 0.157$). All females' progesterone decreased during incubation (Fig. 2C). Throughout nesting and hatching there were no differences between successfully breeding family females and unsuccessful paired females. This, however, might be due to low sample sizes in the family females (*ph*4; $n = 3$; *ph*5: $n = 1$; *ph*6: $n = 2$). In the only successfully breeding female sampled during the first half of incubation, progesterone decreased from 3.64 ng progesterone / g feces during laying (*ph*4) to 0.92 ± 0.69 ng progesterone / g feces at incubation (mean of three samples ± SE per *ph*5) and increased again in the phase preceding hatching (*ph*6: 1.76 ng progesterone / g feces).

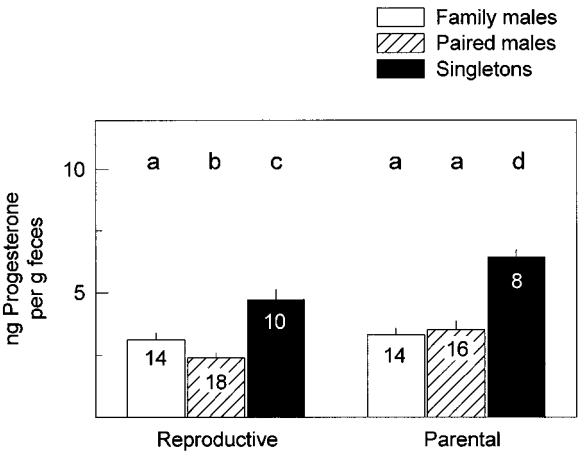

FIG. 4. Mean ± SE fecal progesterone per reproductive (*ph*1 to *ph*6) and per parental season (*ph*7 to *ph*13) in family males (open bars), in paired males without offspring (hatched bars), and in singleton males (black bars). Progesterone in family males did not differ between seasons, whereas in both other male categories levels were elevated during the parental season. Numbers indicate *n* individuals per season; different letters (a, b, c) represent significant differences ($P < 0.05$) between social categories and between seasons.

Levels in the family females increased in October (*ph*9 vs *ph*10: $Z = -2.52$, $n = 8$, $P = 0.023$, Bonferroni corrected), while in paired females without offspring progesterone only slowly increased: the first (marginally significant) increase compared with Summer levels was in January (*ph*9 vs *ph*13: $Z = -2.20$, $n = 6$, $P = 0.055$, Bonferroni corrected; Fig. 2C). Although ANOVA did not indicate an effect of category, progesterone in family females significantly exceeded progesterone in paired females during Summer and in December (Fig. 2C). Furthermore, the family females' mean progesterone of all parental phases (*ph*7–*ph*13) was significantly higher than mean 'parental' progesterone of the paired females without offspring ($Z = -2.39$, $n = 14$, $P = 0.017$; Fig. 5).

DISCUSSION

The present data suggest four major interacting aspects between steroid hormones and social behavior.

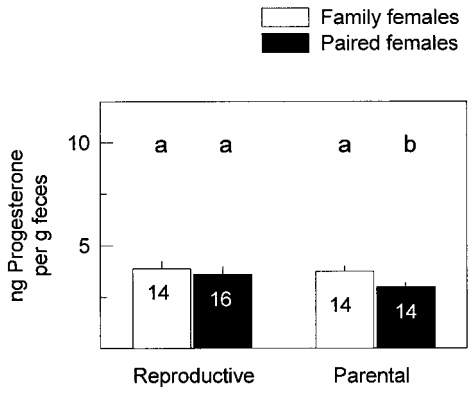

FIG. 5. Mean ± SE fecal progesterone per reproductive (*ph*1 to *ph*6) and per parental season (*ph*7 to *ph*13) in family females (open bars) and in paired females without offspring (black bars). During the parental season family females had higher progesterone than paired females without offspring. Numbers indicate *n* individuals per season, different letters (a, b) represent significant differences ($P < 0.05$) between social categories and between seasons.

(1) During the sexually active season the period of elevated androgen was prolonged in unpaired male geese. Thus, pairing status seems to play a critical role in the individual timing of androgen patterns. (2) The increased estrogen in females without nesting success began earlier in the year, was higher in amplitude, and remained elevated for a longer period of time than estrogen in successfully breeding females. Therefore, the timing of estrogen may be a predictor of female reproductive success. (3) Throughout the year, androgen and progesterone in singleton males exceeded levels in both paired male categories. Androgen and progesterone in male geese may, therefore, be related to pairing status rather than to parenting. In family females, however, during parental phases levels of both hormones were higher than in paired females without offspring. Thus, the relationship of these hormones to behavior seems to be sexually dimorphic. (4) The zenith of fecal androgen, estrogen, and progesterone in singleton males in October and November may indicate a Fall period of increased sexual motivation in unpaired male geese.

Mating and Nesting Phases

As expected, maximal androgen levels coincided with the peak of mating in males and females (Bluhm, 1988; Carter, 1992; Furr and Thomas, 1970; Johnson, 1986; Temple, 1974). Whereas all paired males exhibited a relatively brief androgen peak, levels in unpaired singleton males remained elevated throughout the mating and nesting phases (Fig. 1A). Our data are similar to those found in unpaired male Canada geese (*Branta canadensis moffitti*) which had higher testosterone levels compared with nesting, paired males during the reproductive season (Akesson and Raveling, 1981). These authors concluded that increased testosterone secretion may be more dependent on environmental cues than stimulation provided by the mate. The fluctuations of androgen in male greylag geese outside the reproductive season suggest another possible conclusion: whereas in both paired male categories, androgen was higher during the sexually active than during the parental season (Table 2), there was no such pattern in the singleton males (Fig. 1A). The singletons' prolonged period of high androgen and the additional Fall peak may, therefore, not be related to nonspecific environmental stimuli as suggested by Akesson and Raveling (1981). It may rather be specifically related to the lack of social stimuli provided by a (nesting) female partner (Hausberger and Black, 1990; Silverin, 1990; Wingfield *et al.*, 1990) and indicate the readiness of singletons to pair as soon as there is opportunity. Comparison between androgen patterns of the female categories did not indicate differences during the mating and nesting phases (Fig. 2A); however, no unpaired females were available to compare with in the year sampled.

Estrogen patterns during egg laying in female birds are well known (Akesson and Raveling, 1981, 1984; Bluhm, 1988; Dawson, 1983; Farner and Wingfield, 1980). Fecal estrogen equivalents in female greylag geese were maximal prior to laying and estrogen and progesterone remained elevated throughout laying. This is in agreement with the hypothesis that increased progesterone from mature ovarian follicles induces nesting behavior in female birds (Dawson, 1983; Sharp and Lea, 1996). As in other birds (Dawson, 1983; Dick *et al.*, 1978; Donham, 1979; Harvey *et al.*, 1981; Sharp *et al.*, 1988; Sockman and Schwabl, 1998; Wingfield and Farner, 1978b), the transition to incubation was associated with decreased estrogen and progesterone in all greylag females studied (Fig. 2). These reduced ovarian steroids stem from the cessation of ovulation, ovarian regression, and a suppressive effect of in-

creased prolactin on LH secretion (El Halawani and Rozenboim, 1993; Silver, 1990; Stokkan and Sharp, 1990).

Hatching, Molt and Summer Photorefractoriness

Adults start molting approximately 2 weeks after hatching of the goslings (unpubl. obs.). In agreement with the literature (Dittami and Hall, 1983; Péczely, 1992; Payne, 1972), fecal androgen and estrogen equivalents were at a minimum during molt in both sexes. This may reflect the seasonal separation of breeding and molting in temperate-region birds (Assenmacher and Jallageas, 1980; Wingfield and Farner, 1980).

Within both family and paired females, fecal androgen was higher during the reproductive than during the parental season (Figs. 2A and 3). Low androgen levels throughout Summer are also characteristic of long-day refractoriness (Farner and Wingfield, 1980; Paulke and Haase, 1978; Péczely *et al.*, 1993; Péczely and Dong Xuan, 1995). However, in contrast to patterns of plasma testosterone in female barheaded geese (*Anser indicus;* Dittami, 1981), greylag females with goslings had higher fecal levels of androgen than paired females without offspring (Fig. 3). Only throughout Summer and Fall did the family females exhibit testosterone-dependent aggressive behavior (Dittami, 1981; unpubl. data) in within-flock competition for resources (Schneider and Lamprecht, 1990; Sedinger and Raveling, 1990). Therefore, it is suggested that high androgen in family females is part of their parental investment, no matter whether this is cause or effect.

There was no such difference between family males and paired males without offspring (see above). Thus, the present data do not invoke androgen in parenting of male geese (Lamprecht, 1992; Schneider and Lamprecht, 1990; Sedinger and Raveling, 1990), which contrasts with the phenomenon that androgen interferes with parental care in males of altricial species (Dittami *et al.*, 1991; Hegner and Wingfield, 1987; Ketterson *et al.*, 1992; Saino and Moller, 1995; Schoech *et al.*, 1998; Silverin, 1990).

Mean progesterone was higher outside the sexually active phases than during the reproductive season within paired males without offspring and singletons, whereas within the family males there was no such pattern (Fig. 4). If progesterone (and androgen) was of gonadal origin, this could be explained by regression of the testes in family males as they rear goslings, but much longer maintenance of mature testes in the singletons. In contrast, family females had higher progrestone during parental phases than paired females without offspring (Fig. 2C), indicating a positive interaction between progesterone and parental behavior in female geese but not in males, as suggested for androgen.

Fall and Winter Phases

Fall peaking of LH and androgen, as described in males of a number of birds (Boswell *et al.*, 1995; Campbell *et al.*, 1978; Donham, 1979; Paulke and Haase, 1978; Péczely *et al.*, 1993) was not always accompanied by increased testis weight or courting behavior (Balthazart and Hendrick, 1976; Dittami, 1981; Lincoln *et al.*, 1980). However, if Fall courting occurs (e.g., in mallards) increased androgen may be a consequence of pair formation (Dawson, 1983).

In October, the unpaired greylag males' fecal progesterone was double that of females and was significantly above both paired male categories. This coincided with the Fall peaks of androgen and estrogen in these males (Fig. 1). Increased androgen secretion in Fall and Winter may have a preparatory role for reproduction and sexual behavior during the early phases of the next mating season (Lincoln *et al.*, 1980). Another function of this phenomenon may be the regulation of social interactions or of intense competition for food (Kotrschal *et al.*, 1993; Rohwer and Wingfield, 1981).

Some role for androgen, estrogen, and progesterone in Fall and Winter fattening of migratory birds cannot be ruled out. Vernal hyperphagia and fattening was suggested to require sex steroids prior to the period of photostimulation (Deviche, 1994). This, however, does not explain why Fall peaks of androgen, estrogen, and progesterone in greylag geese were found only in singleton males and not in any other social category (Fig. 1). It may indicate some potential of unpaired male geese for sexual behavior in the Fall corresponding with seasonal increases in courting behavior (Dittami, 1981). Taken together, the singletons' prolonged period of high androgen levels (throughout Fall and

Spring phases) may point at a year round increased need for competitive ability in unpaired males, resulting in readiness for action whenever a female might become available. The difference between the steroid patterns of both paired male categories and of singletons once more indicates that in geese the social stimulation by a permanent partner is required to produce the distinct seasonal hormonal profile.

Fecal estrogen in unsuccessful greylag females increased earlier than in successful ones (starting in December) and remained higher in amplitude throughout the year (Fig. 2). Several hormones (insulin, glucagon, corticosteroids, thyroid hormones, prolactin) are involved in avian fat deposition, the liver being the major site of lipogenesis. Additionally, an action of estrogen on hepatic fatty acid metabolism has been suggested (Griffin, 1993). Female ptarmigans (*Lagopus lagopus*), for example, that succeeded in raising a brood stayed leaner longer than those that did not and Fall fat deposition in the brood-raising females began later (Stokkan and Sharp, 1990). In breeding female Canada geese, annual fluctuations of body weights and estrogen were positively correlated, whereas nonbreeding females showed comparatively small seasonal variation in body weights (Akesson and Raveling, 1981). In penguins (*Spheniscus magellanicus*), females whose nests failed tended to have higher estradiol-17β compared with successful females (Fowler *et al.*, 1994). However, it remains to be tested whether the timing of estrogen is indeed involved in Fall fattening and/or breeding success in geese. A female would then more likely be successful when maintaining elevated estrogen within a critical range of magnitude over a short time window.

In conclusion, the seasonal patterns of fecal steroids revealed that unpaired individuals retained a hormonal state closer to sexually active phases throughout the year than paired geese. Within both paired males the seasonal hormone profiles clearly differed between a sexually active and a parental season. In the Fall, only singleton males showed peaks of androgen, progesterone, and estrogen that may have derived from extra-gonadal sources. An involvement of androgen and progesterone in male parenting of precocial birds was not apparent. However, in the females both hormones seemed to be positively related to parental behavior.

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