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Circannual Plasma Androgen Levels in Free-Living Male Great Tits (*Parus major major* L.)

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Seasonal variations of plasma androgen levels in free-living adult and juvenile male Great Tits (*Parus major major* L.) were measured by radioimmunoassay over a period of 5 years. In 4 of the 5 years a peak of androgen concentrations was observed during the breeding season (in 1 year during the prebreeding season, followed by a nadir during winter. Besides these circannual fluctuations, considerable variations in plasma androgen levels between comparable behavioral periods of successive years were noticed (187 to 18.5 ng/dl during the breeding season and 86 to 16 ng/dl during winter). No relationship was found between these variations and possible causative factors such as age and origin of the birds and the time of trapping and blood sampling during the day. The large between year variations in androgen levels may be caused by changes in the birds' environment including density of the population and the availability of food. Additional data are required to test this hypothesis.

During the last few decades a great number of studies have been carried out on the seasonal variations of androgen concentrations in birds (Donham, 1979; Jallageas and Assenmacher, 1980; Paulke and Haase, 1978; Schwabl *et al.*, 1980; Silver *et al.*, 1979; Stokkan and Sharp, 1980; Wingfield *et al.*, 1978; Temple, 1974).

In several of these studies changes in androgen levels are compared with behavioral and/or histological parameters (Dittami, 1981; Kerlan and Jaffe, 1974; Lincoln *et al.*, 1980; Stokkan and Sharp, 1980; and Wingfield *et al.*, 1980). Many of the data concern birds in captivity while few data are available on free-living Passerines. Nevertheless, several characteristics of Great Tits (*Parus major major* L.) such as clear differences in plumage between adult and juvenile birds and in secondary sex characteristics, a preference for breeding in nestboxes, and the easy trapping of the birds render them advantageous subjects for this type of study. Furthermore their changing behavior and population dynamics are quite well understood (Dhondt, 1970; Hinde, 1952; Kluyver, 1951; Lack, 1954; Perrins, 1979).

The annual cycles in plasma levels of testosterone and 5α-dihydrotestosterone (DHT), as well as seasonal changes in the ultrastructure of Leydig cells and the size of the testes in free-living Great Tits were reported by Silverin (1978) and Röhss and Silverin (1983). In their studies the authors assumed that the one annual cycle studied was typical for all years.

In a long-term study of the Great Tit at Ghent, Belgium, we examined the variations of plasma androgen levels in free-living male birds over a period of 5 years. We analyzed the relationship between the observed fluctuations in plasma androgen levels and possible causative factors such as age and origin of the birds and the time of trapping during the day. We also com-

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pared the observed variations with changes in the composition and the behavior of the bird population.

MATERIALS AND METHODS

1. Population and Habitat

Between March 1977 and February 1982 a total of 728 blood samples were taken from free-living male Great Tits (*P. major major* L.) in a 27 ha mature beech wood (*Fagus silvatica*) with an understory of *Rhododendron praecox*, in which a surplus of Great Tit nestboxes have been available since 1964. This area supports a large population of Great Tits during winter, a major proportion of which are immigrant juveniles. The average breeding density is 11 pairs/10 ha compared to 23 pairs/10 ha in an optimal oak woodland and 14 pairs/10 ha in a coniferous woodland with a rather rich undergrowth (Dhondt, 1970).

All individuals were banded with a metal ring of the Belgian Ringing Scheme and two or three color bands. This allowed us to follow the temporal pattern of plasma androgen levels in individuals. After capture the age of the birds was noted, as was their origin and status, e.g., adult resident birds, autochthonous juveniles (all nestlings in the study area were banded when 15 days old), and allochthonous juveniles, i.e., juveniles immigrated in the study area mainly before winter. We consider as juvenile a bird prior to and during its first breeding season; consequently a bird is considered adult after its first breeding season.

2. Bird Trapping and Blood Collection

Trapping methods varied according to the season and related differences in animal behavior. Five behavioral periods were studied.

Winter. From the end of October until the end of February, nestboxes were checked during bimonthly evening visits. Roosting male Great Tits were captured and taken to the laboratory because blood could not be sampled in the field. Birds were transported in darkened cages containing four to six animals (period: 1977–1979) or only one animal (from October 1979 onward). Between 1 and 4 hr after arrival in the laboratory, blood samples were collected from the jugular vein with a heparinized microhematocrit capillary tube, after puncture of the blood vessel with a 23-gauge needle.

Prebreeding season. During the second half of March, characterized by intense spring territorial behavior, blood was obtained as in winter time.

Breeding season. During May and June, adult male birds were trapped upon returning to their nest to feed first-brood nestlings, aged 10–12 days and placed in individual darkened cages until blood was collected in the field, i.e., within 10 min after capture. Heparinized capillary tubes, as described for the winter, were used.

Summer and autumn. From July until the end of October, Great Tits were mistnetted in daytime and blood was collected as in the breeding season. Males were divided into a group of moulting birds (summer, July–August) and a group of moulted birds (autumn, August–October).

3. Radioimmunoassay (RIA) Method

Antiserum. We generated testosterone antiserum in rabbits by multiple intradermal injection of the antigen conjugate testosterone-3-carboxymethyl oxime: bovine serum albumin, 1 mg in a stable emulsion of water and complete Freunds adjuvant (Vaitukaitis *et al.*, 1971). Antisera with high titer were studied for cross-reactivity with heterologous steroids as described by Abraham (1969). The antiserum used in the present study had a titer of 72,000; it was highly specific and, of eight steroids tested, only 5α-dihydrotestosterone showed significant cross-reactivity. Cross-reactions at the 50% inhibition level (Abraham, 1969) were: testosterone, 100%; 5α-dihydrotestosterone, 28%; 5α-androstane-3α, 17β-diol, 5%; 5-androstene-3β,17β-diol, 0.45%; androstenedione, 0.34%, dehydroepiandrosterone, 0.05%; progesterone, 0.03%; estradiol-17β; and cortisol, less than 0.01%.

Aliquots of the antiserum, diluted fivefold with Tris-buffered saline (TBS, 50 m*M* Tris, pH 8.0, at room temperature, 0.1% sodium azide, and 0.1% gelatin), were stored frozen at −20°. Under these conditions the antiserum remained stable for over 7 years.

RIA. Plasma samples, from 0.05 to 0.2 ml, were brought up to 0.5 ml with TBS and were then extracted with 5 ml of diethylether (Carlo Erba, Milano, Italy). The extract was dried under a gentle stream of nitrogen, the residue was dissolved in 0.5 ml of absolute ethanol (Merck AG, Darmstadt, FRG), and duplicate 0.2-ml aliquots were dried in disposable glass tubes. For calibration curves duplicate aliquots containing 5 to 200 pg of testosterone standard (Sigma Chemical Co., St. Louis, Mo.), dissolved in absolute ethanol, were dried in disposable glass tubes. To the dry residues of samples and standards 0.1 ml of antiserum, diluted 1:36000 in TBS, was added. The contents of the tubes were mixed well and incubated 45 min at 37°. [^{3}H]Testosterone (20000 dpm, 100 Ci/mmol, Amersham International Ltd., Amersham, Bucks., U.K.), were added and incubation was continued overnight at 4°. Dextran-coated charcoal suspension (0.5% Norit A, 0.05% Dextran T70), 0.5 ml, was added and the tubes were incubated for 30 min at 0°, with intermittent shaking on a Vortex mixer. The tubes were centrifuged (10 min, 2000*g*, 4°) and the supernate was decanted into counting vials containing 3 ml of liquid scintillation cocktail. The radioactivity in the

bound fraction was counted for 5 min in a Packard Model 3255 beta counter with 40% efficiency (De Boever *et al.*, 1983). In each assay a buffer blank and control plasma pools were included. Since the extracted plasma samples were assayed without purification and since the antiserum reacts appreciably with both testosterone and 5α-dihydrotestosterone, measurements are referred to as androgen concentration.

Characteristics of the RIA

The efficiency of extraction of testosterone from the plasma samples was determined by measuring the recovery of added [^{3}H]testosterone. This recovery averaged 90.0 ± 4.5% (SD) in 65 RIAs performed during a period of 18 months (1976–1977) and did not change in subsequent assays. In the same 65 RIAs, sensitivity of the assay defined as the least amount of testosterone that could be distinguished from zero (mean ± 2 SD) (Midgley *et al.*, 1969) averaged 21 pg/ml plasma. Buffer blank values were generally below 10 pg/ml. Both sensitivity and buffer blank values did not change significantly thereafter.

As a measure of accuracy different pools of male Great Tit plasma were diluted with TBS, and 0.5 ml of each dilution containing 10 to 150 μl plasma, were assayed as described. The regression of the measured (y) and of the expected (x) androgen concentrations in 30 diluted samples was linear, $y = 1.00\,x - 0.04$, and the correlation coefficient was $r = 0.993$. Different amounts of testosterone standard (10 to 200 pg) were added to 0.2-ml aliquots of pooled Great Tit plasma (with low androgen levels) and the analytical recovery of testosterone was determined. With different plasma pools analyzed on different occasions between 1976 and 1981, the mean recoveries varied between 97.9 and 102%. In the same period small tests of accuracy were performed regularly by determining the analytical recovery of 25 pg of testosterone added to 0.1 and 0.2 ml of Great Tit plasma samples, containing between 8 and 40 ng androgen/dl. This recovery varied between 94 and 102.5%.

Precision of the assay was evaluated in terms of intra- and interassay coefficients of variation, CV. The intraassay CV of 10 replicate determinations in duplicate within one assay of two different control pools of Great Tit plasma was 6.2% for a mean androgen concentration of 52 ng/dl and 5.1% for a mean androgen concentration of 96.7 ng/dl. The same control plasmas were reassayed 6 months later under identical conditions. Mean CVs and androgen concentrations were 6.1% for 51 ng/dl and 6.4% for 97.2 ng/dl, respectively. The interassay CV was obtained by measuring androgen concentrations in different control pools over longer periods of time. Sixty-five radioimmunoassay determinations of both control plasma pools, over a period of 18 months, were performed with interassay CVs of 9.3 and 7.3% for mean androgen concentrations of 51.2 ng/dl and 98.6 ng/dl. Both intra- and interassay CVs for all control plasma pools used over the period 1977–1982 were calculated per calendar year and results were expressed as year to year variations of the CVs of both high and low androgen levels.

4. Statistical Analysis

We calculated levels of significance using a Mann–Whitney U test and a Kruskal–Wallis analysis of variance (Siegel, 1956).

RESULTS

1. Reliability of the RIA over Several Years

As stated earlier (see "Characteristics of the RIA") neither the efficiency of extraction of the plasma samples with diethylether nor the minimum detectable dose and the recoveries of added testosterone to plasma samples were subject to significant changes over the period 1977–1982. The absence of important variations of the accuracy with time is exemplified in Fig. 1 presenting the position and shape of six calibration curves, spread over a 5-year period, from February 1977 to June 1981. The B/B_0 values of the six curves are similar for each of the testosterone concentrations and the 50% B/B_0 values correspond to a mean testosterone concentration of 30.4 (SD 2.5) pg. These calibration curves were used to calculate results of the following six periods: prebreeding season 1977; breeding season + summer 1978; winter 1978–1979; winter and prebreeding season 1980; summer 1980; prebreeding and breeding season 1981 (see Fig. 3). The year to year variation of intra- and interassay CVs of different control plasma pools are presented in Fig. 2. The pools ($N = 15$) were divided into two groups according to their androgen levels: nine low (25 to 55 ng/dl) and six high level (80 to 120 ng/dl) pools.

2. Time of Blood Sampling and Plasma Androgen Levels

During the breeding season, summer and

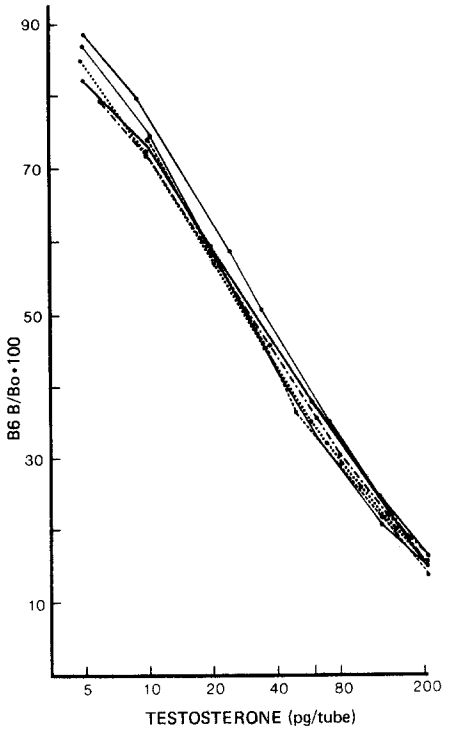

FIG. 1. Calibration curves for measurement of testosterone over a 5-year period. See Results, section 1 for explanation.

autumn period birds were not always trapped at the same time of the day. The mean plasma androgen levels observed at different times of the day are shown in Table 1. For all periods of the year analyzed (N = 12), no statistically significant differences in plasma androgen levels depending on the time of blood sampling during the day were observed.

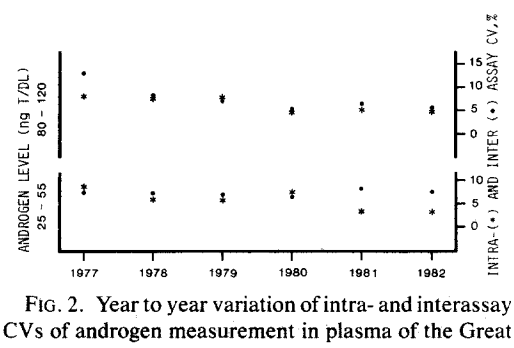

FIG. 2. Year to year variation of intra- and interassay CVs of androgen measurement in plasma of the Great Tit. See Results, section 1 for explanation.

3. Age and Origin of the Birds and Plasma Androgen Levels

Figure 3 presents mean plasma androgen concentrations during 25 consecutive periods. For 4 periods data of only one group, either adult or juvenile are available. For the other 21 periods data of both age groups, juvenile and adult male Great Tits are shown. Since statistically significant differences between androgen levels in both age groups were not observed, data of both age groups were pooled for further studies.

During the autumn period juvenile male birds of different origin could be trapped: occasionally immigrant birds, trapped only once (allochtonous) and birds that were born in the area under study (autochtonous). Their plasma androgen levels were not significantly different (Table 2).

4. Seasonal Variations of Plasma Androgen Levels

The circannual changes in the plasma androgen levels of male Great Tits during 5 consecutive years, are shown in Fig. 3. For each of the 5 years studied, considerable fluctuations of androgen levels between the behavioral periods, i.e., circannual changes were observed. In addition considerable differences were also noted between equal behavioral periods over successive years. In 4 out of 5 years maximal androgen levels were found during the breeding season and the actual level reached varied by an order of magnitude between years: the highest average being 187.06 + 17.58 ng/dl in 1979 compared to 18.44 ± 8.53 ng/dl in 1980. After the maximum in the breeding season, androgen levels decreased toward winter, although the lowest averages were not always found during winter. Androgen levels in winter also varied between years, e.g., 16.74 ± 2.80 ng/dl in 1979–1980 compared to 86.74 ± 7.54 ng/dl in 1978–1979. The highest winter androgen level was therefore higher than the lowest breeding season androgen level.

All within year circannual differences ob-

TABLE 1
PLASMA ANDROGEN LEVELS DURING DIFFERENT PERIODS AND TIMES OF BLOOD SAMPLING IN FREE-LIVING MALE GREAT TITS

Period of the year	Mean androgen concentrations in ng/dl plasma and (number of samples analyzed) at different times (hr) of blood sampling during the day				Statistical evaluation[a]
	8–10	>10–13	>13–16	>16–21	
Breeding season					
1977		41.4 (2)	19.7 (3)	25.5 (11)	NS
1978		166 (2)	112.7 (3)	120.2 (13)	NS
1979			309 (1)	180.6 (11)	
1980	4 (3)	25.1 (7)	25.3 (3)	4. (4)	NS
1981	131 (3)	166 (6)	81.5 (4)		NS
Summer					
1977		10.7 (1)	11.3 (1)	15.4 (2)	NS
1978	62.3 (3)				
1979	110.2 (10)	50 (4)	94 (1)		NS
1980	86 (5)	19.8 (17)			NS
1981	31 (2)	21 (1)			
Autumn					
1977	78 (1)	11.2 (2)	12 (1)		NS
1978	87.7 (3)				
1979	33.4 (11)	35.9 (16)	37.5 (7)	27.5 (4)	NS
1980	18 (5)	14.5 (13)	20.4 (19)	17.5 (8)	NS
1981	80.1 (8)	71.8 (26)			NS

[a] Kruskal–Wallis analysis of variance or Mann–Whitney U test.

served for the 5 years and all between year differences for the five behavioral periods were significant (Table 3). These striking interyear fluctuations in the mean plasma androgen levels are reflected in individual birds. Patterns of individual plasma androgen levels over five breeding seasons and five winters are presented in Figs. 4a and b.

5. *Fluctuation of Androgen Levels and Composition of the Population*

The composition of the population over different periods and years is summarized in Table 4. Breeding densities fluctuated between 7.8 pairs/10 ha in 1977 and 11.5 pairs/10 ha in 1980. After peak levels during the breeding season, plasma androgen concentrations decreased during the moult of adult and juvenile birds. The degree over which these levels decreased was subject to considerable fluctuations. Over the 5-year period ($N = 5$) studied a weak relationship ($r_S = 0.700$ $P < 0.2$) was found between the number of young fledged and the decrease in androgen levels. On the other hand no significant relationship (but a negative trend) was found between the percentage of surviving juveniles and decreasing androgen levels ($r_S = -0.500$ $P > 0.1$). From the summer to the autumn period androgen levels increased in 3 years with, respectively, 19, 23.4, and 80.6% and decreased in 2 years with, respectively, 15 and 58.8%. No significant relationship ($r_S = -0.500$, $P > 0.1$) but a negative trend was found between the cumulative number of birds trapped and the average androgen level.

An estimation of birds densities during the winter, based on regular tours of the study area, observations at a feeder, and bimonthly evening controls revealed fluctuations between 31 and 69 individuals per 10 ha (Table 4). From the breeding season to the winter androgen levels decreased in 4 out of 5 years. A weak relationship was

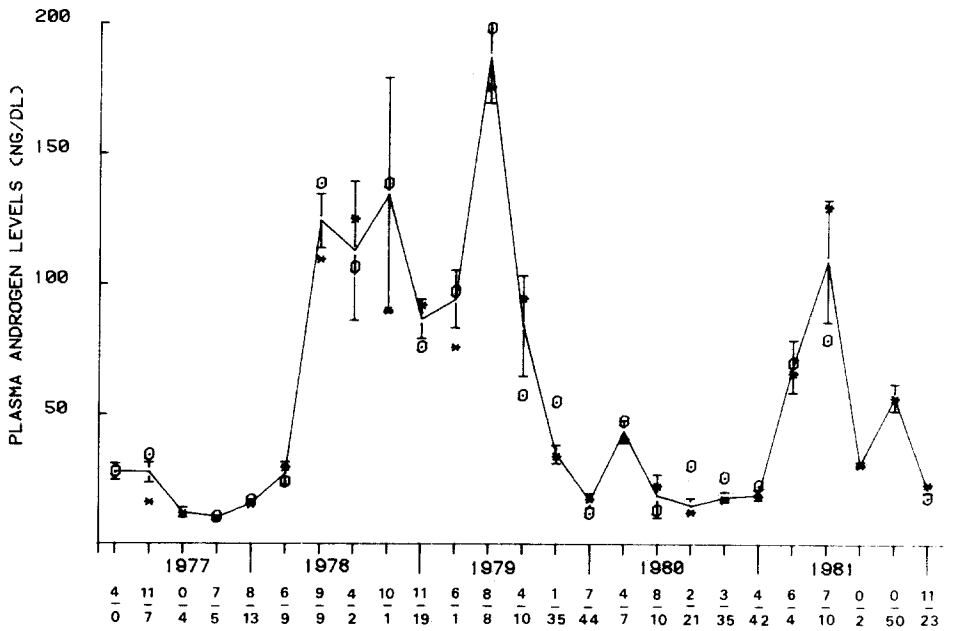

FIG. 3. Seasonal variations of plasma and androgen levels in free-living adult (⊙) and juvenile (*) male Great Tits. Mean and SEM (vertical bars) values in ng/dl. Numbers of adult and juvenile birds are shown below each period.

found between the winter density and the drop in androgen levels between breeding season and winter ($r_S = 0.800$ $P < 0.1$).

DISCUSSION

Our results show marked circannual variations of plasma androgen levels in free-living male Great Tits. These results are in agreement with earlier reports in different taxa of animals including birds. Peak levels are often found during spring when territorial behavior is most intense (Dittami, 1981; Jallageas and Assenmacher, 1974; Lincoln *et al.*, 1980; Paulke and Haase, 1978; Temple, 1974). Röhss and Silverin (1983) found testosterone peak levels in March for free-living male Great Tits in Southwest Sweden. We found peak levels of androgens during the breeding season (May–June) but no significant differences

TABLE 2
PLASMA ANDROGEN CONCENTRATIONS[a] OF AUTOCHTHONOUS AND ALLOCHTHONOUS JUVENILE MALE GREAT TITS, TRAPPED DURING AUTUMN

	Autochthonous			Allochthonous			
Year	Mean	(SE)	*N*	Mean	(SE)	*N*	Statistical evaluation[b]
1979	28.9	(2.8)	9	28.9	(5.6)	13	NS
1980	20.3	(3.8)	14	18.6	(2.6)	12	NS
1981	44.5	(7.0)	8	41.4	(7.1)	26	NS

[a] Values are expressed as ng/dl.
[b] Mann–Whitney *U* test.

TABLE 3
WITHIN AND BETWEEN YEAR VARIATIONS OF ANDROGEN LEVELS[a] IN FREE-LIVING MALE GREAT TITS

Within year variation[a]			Between year variation[a]		
Year[b]	χ^2	*P*	Period	χ^2	*P*
1977	35.43	<0.01	Prebreeding	33.05	<0.01
1978	36.44	<0.01	Breeding	60.71	<0.01
1979	77.96	<0.01	Summer	29.98	<0.01
1980	28.22	<0.01	Autumn	66.42	<0.01
1981	42.20	<0.01	Winter	79.46	<0.01

[a] Kruskal–Wallis ANOVA.
[b] One year is the period from March 1 up to February 28 (e.g., 1977 = 03.01.77–02.28.78).

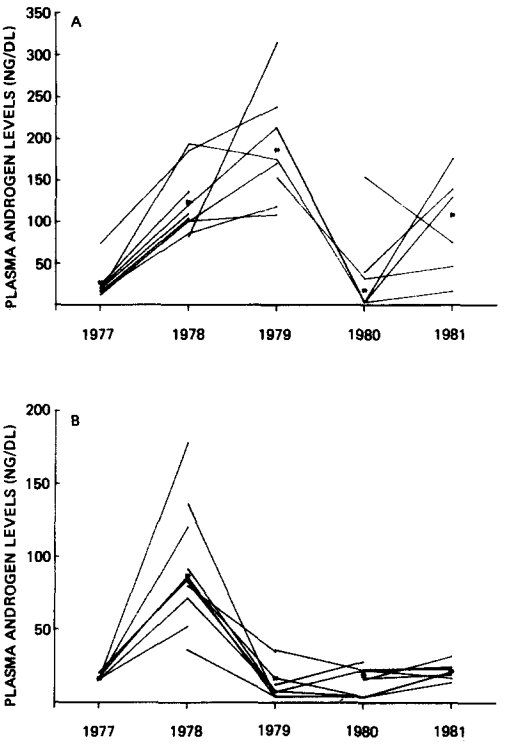

FIG. 4. Variation of plasma androgen levels (ng/dl) in individual male Great Tits over (a) different breeding seasons and (b) different winters, compared with the mean (*) plasma androgen levels in the population.

were found when the data of April and May were compared (respectively, 126 ± 39.1 ng/dl and 100.1 ± 23.6 ng/dl) which means a delay of 1 month in relation to the data of Southwest Sweden. Annual changes were compared with testes size (Silverin, 1978) and with changes in the ultrastructure of Leydig cells (Röhss and Silverin, 1983). In the Swedish population an age related difference in androgen levels was only found in 8% of all cases (September) with higher levels for juveniles. We found only in 5% of all cases, i.e., one period (breeding season 1977) out of 21 periods an age-related difference with higher androgen levels for adults. Based on the 5% level this difference could be neglected and suggests that also the age-related difference in the Swedish Tits may be due to chance. If subsequent annual cycles of plasma androgen levels in free-living male Great Tits, were compared over a 5-year period, significant differences between equal behavioral periods were observed. These differences are both striking and unexpected since to our knowledge no comparable literature references are available. Jallageas and Assenmacher (1983) studied androgen levels of captive male dormouse (*Glis glis*) over a 2-year period. During the first year peak levels were found in June–July. A fairly similar annual pattern was found during the second year except that the rise in plasma testosterone levels was delayed by 1 month and maximal levels were significantly lower (−59%) than those measured in the preceeding year. Jallageas and co-workers suggested that the decreased amplitude would not be caused by external factors but rather by a prolonged captivity of the animals. Paulke and Haase (1978) studied testosterone levels in wild Mallard drakes over 2 years with different determination methods (CPB and RIA). In the first year peak levels were found but lower (−58%) testosterone concentrations. According to the authors this was probably due to different techniques and not to different breeds.

The striking within and between year fluctuations in androgen levels reported in this study were not due to changes in methodology nor to fluctuations in assay performance. As a matter of fact RIA characteristics such as sensitivity, buffer blank values, accuracy, and assay precision did not vary more than 10% over the 5-year period studied.

It is difficult to explain the fluctuating androgen levels through differences between the individuals sampled because (1) for each period studied birds differing in age or origin had comparable androgen levels and (2) marked individuals that were followed over several seasons showed androgen values varying in parallel to the overall population levels.

According to Balthazart (1976) testosterone levels in domestic ducks (*Anas pla-*

TABLE 4
CIRCANNUAL CHANGES IN THE COMPOSITION OF THE FREE-LIVING MALE GREAT TIT POPULATION, IN ANDROGEN CONCENTRATIONS AND TEMPERATURES OVER 5 YEARS

	Breeding season			Summer		
	Density /10ha	Androgen conc (ng/dl)	Temp °C	Number of young fledged	Variation of androgen conc (%)[a]	Temp °C
1977	7.8	27.4	12.4	150	−56.7	16.7
1978	9.6	124.1	13.0	151	−9.2	16.1
1979	9.6	187.1	12.6	196	−55.1	16.6
1980	11.5	8.4	12.8	223	−19.9	16.8
1981	11.1	109.1	13.8	131	−71.6	17.2

	Autumn					Winter				
	Cumulative number trapped			Androgen conc	Temp	Density /10 ha			Variation of androgen	Temp
	♂	♀	Tot	(ng/dl)	°C	♂	♀	Tot	conc. (%)[a]	°C
1977	131	198	329	10.1	12.1	91	59	150	−42.40	4.4
1978	48	87	135	134.1	11.5	70	56	126	−30.1	2.1
1979	110	158	268	34.6	11.8	59	60	119	−86.6	4.6
1980	208	336	544	18.2	9.3	95	92	187	+3.70	3.3
1981	209	317	526	56.9	9.2	48	36	84	−80.30	3.7

[a] As compared to androgen conc (ng/dl) in the breeding season.

tyrhynchos) showed a sharp decrease between 8 and 10 AM followed by a small but not significant increase in the afternoon. In our data the highest androgen levels were mostly found between 8 and 10 AM (Table 1) but no significant differences were found with other times of the day while important individual variations occurred as indicated by the relative large standard errors.

Finally we must accept the observed in between year variations as real and probably caused by changes in the birds' environment. These variations may originate in differences in feeding conditions, differences in population density or differences in the climatic conditions. The influence of climatic circumstances appears rather unlikely. During the winter 1978–1979 mean environmental temperatures decreased from 6.1 and 3.3° in November and December 1978 to −2.4 and 0.4° in January and February 1979. The proportion of winterdays (max. temp. below zero) increased from 13% in November–December 1978 to 25% in January–February 1979. These rather drastic changes in climatic conditions caused no changes in the observed androgen values (respectively, 90.3 ± 51.9 ng/dl, 91.6 ± 44.6 ng/dl, and 85.8 ± 37.0 ng/dl, 97.4 ± 30.8 ng/dl). Also Wingfield *et al.* (1982) found little effect of environmental temperature on plasma levels of testosterone in his experimental White-crowned Sparrows (*Zonotrichia leucophrys*). The most obvious difference between years is, in fact, caused by an interplay between all these factors: in some years large numbers of birds compete for limited food resources, whereas in other years food is abundant but summer density low.

Flickinger (1961) found that the adrenals of chicks kept at high density levels were heavier than the adrenals of birds kept at low density. He further reported (Flickinger, 1966) that the steroid output stimulated by ACTH caused a suppressive effect on gonadotropin secretion. According to Lewis *et al.* (in Wingfield *et al.*, 1982) se-

vere and chronic restriction of food for at least 24 hr caused plasma corticosterone to increase and testosterone to decrease in White-crowned Sparrows (*Z. leucophrys gambelli*). Our results give some indications for an inverse relationship between androgen levels and population density. For the winter period we found a marked decrease of plasma androgen values at higher winter densities and also during the summer period androgen levels decreased more when more young fledged and survived. This suggests a possible inverse relationship between corticosteroids and androgens. In fact this is a well-known phenomenon in men where an acute decrease in serum testosterone is observed in association with a variety of stressful conditions (Dessypris *et al.*, 1976; Cartensen *et al.*, 1972) and with elevated cortisol levels of exogenous or endogenous origin (Cumming *et al.*, 1983). Similarly we would expect to find decreased androgen values in birds subjected to conditions of stress and/or enhanced adrenal activity. This would apply to situations characterised by elevated summer population densities (after fledging) and/or poor summer food conditions resulting in high intraspecific competition for space and/or food and therefore a higher adrenal activity.

We have insufficient data on food availability to verify this statistically over the 5-year period. Yet, the situation may be exemplified by the data available for the summers of 1977 and 1978, in which an equal number of young birds fledged. In the summer of 1977 the birds frequently fed from the feeder, whereas this was not the case in 1978. Food conditions therefore, seemed to be better in 1978 compared to 1977 and intraspecific competition (and stress) between birds must have been less. Concomitantly plasma androgen levels in the summer of 1978 by far exceded those of the summer period 1977. On the other hand postfledging mortality can vary considerably and may have great impact on density and behavior of surviving birds. But over the 5 years of our study we could not find a clear correlation between the proportion of surviving juvenile birds and the observed decrease in androgen levels.

In the present situation we can best explain the striking between-year fluctuations in androgen levels by the correlation we observed between these androgen levels and the density of the Great Tit population. However, many more data, especially on the availability of food and on changes in the population density, are required to test our hypothesis of a possible causative relationship between both variables, i.e., density-related or dependent changes in plasma androgen concentrations.

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