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Response of female Great Tits *Parus major* to photoperiodic stimulation and the presence of a male

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Abstract

The response of female Great Tits *Parus major* to photoperiodic stimulation was investigated by measuring the plasma concentration of oestradiol and the growth of the ovary in caged and in free-living birds. Stimuli from the male and from the nest-building labour are assumed to promote the ovaries to complete maturation. Two groups of females were kept on long days (20L:4D), one group consisting of female/male pairs with access to nest-building facilities, and one containing female/female pairs. Another group of female/female pairs was kept on short days (8L:16D). A reference group consisted of free-living females, caught during February–April. The oestradiol concentrations of the caged females on both light regimens remained low and at the same level as that of the free-living females prior to the nest-building. The hormone level peaked only among free-living females during the egg-laying period. The females kept on long day regimen only reached incomplete egg follicle growth which peaked at about the same size as that of the free-living females in the early nest-building period. The presence of a male stimulated neither the oestradiol secretion, nor the ovarian growth.

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Introduction

Internal mechanisms regulating the breeding cycle in birds respond to both photoperiodic stimulation and supplementary environmental cues such as behavioural interactions, availability of nest-sites, nest-building material and food. The seasonal variation in day length determines whether the reproductive system is active or inactive, while supplementary cues may accelerate or retard gonadal development. The photoperiod is registered and measured by photoreceptors and circadian clocks in the hypothalamus. In birds, contrary to mammals, the eyes and the pineal gland do not participate in the registration of light (Murton & Westwood 1977, Jameson 1988, Becker, Breedlove & Crews 1992). Supplementary information may however be conveyed by the eyes. The biological clock determines the timing of the ovulatory cycle and the release of hormones. Thus, birds use their circadian rhythms to initiate breeding in response to increasing day length. The latter is responsible for activation of the reproductive system. The photoperiodic stimulation of the gonadal system is triggered by the simultaneous occurrence of a photosensitive phase of an entrained circadian oscillation and the light part of the photo-cycle (Farner 1975).

At northern latitudes the breeding season for the Great Tit *Parus major* is springtime (April–June). The study period is divided into different reproductive stages during the period February-May.

* The pre-nesting period February/March starts when the males establish their breeding territories.
* The nest-building period starts in mid April. Only the female is occupied in the construction of the nest.
* The egg-laying period starts in late April. Normally the female lays one egg per day.

These periods imply important physiological changes. In the female, the sexual behaviour is dependent on the presence of high levels of gonadal steroids, such as oestradiol and progesterone (Barfield 1971, Meyer 1974).

As the breeding season approaches, the ovary and oviduct develop and increase in both weight and size. The ovary, located dorsally on the left side in the anterior part of the body cavity, contains thousands of oocytes. The majority of these oocytes never develop or degenerate. Thus, only a small number will mature and ovulate. A developing oocyte is surrounded by granulosa cells, which support the oocyte during its development enabling the formation of yolk material. Together, the oocyte and the granulosa cells constitute an ovarian follicle. The development of the follicle continues with the development of surrounding theca layers, which contain cells important for the hormone production.

Only under photoperiodic stimulation can a developing follicle grow to maximal size. When the oocyte is fully developed (preovulatory follicle), ovulation occurs when the follicle wall ruptures. For a more detailed description of ovarian and follicular growth in birds, see King & McLelland (1979).

The aim of this study was to investigate experimentally whether the photoperiodic response of the reproductive organs in female Great Tits needs additional stimulation generated by the presence of a male, a nest-box and naturally occurring nest-building material in order for these organs to reach full maturity.

The progress of maturation was followed by measuring the concentrations of oestradiol in the blood as well as the follicle growth. This was done by keeping female Great Tits on controlled light schedules in the presence and absence, respectively, of a male, nest-box and nest-building material. These laboratory studies were compared with the normal development in free-living birds.

Methods

Experimental design

Great Tits were caught in early February with mist-nets near Göteborg, south-western Sweden. They where placed in cages in three separate rooms, especially designed for photoperiodic experiments, and at a constant temperature and humidity. Before the experiment started, all caged birds were kept on short days for about three weeks. The cages measured 55 x 30 x 40 cm. The birds had a continuous supply of water, sunflower seeds, and dried and living insects.

* Group 1 consisted of eight females and eight males (female/male pairs). The birds were kept one pair per cage, and each cage contained one nest-box and fresh nest-building material. These birds were kept on long days, i.e. 20 h of light and 4 h of darkness (20L:4D).
* Group 2 consisted of eight females, grouped two and two (female/female pairs). These birds had no access to males, nest-boxes or nest-building material, nor could they see or hear the males from Group 1. These birds were kept on long days (20L:4D).
* Group 3 consisted of eight females, grouped two and two (female/female pairs). No males, nest-boxes or nest-building material were available. These birds were kept on short days (8L:16D). This group served as a control group.
* Group 4. Four free-living females were captured each month during different breeding periods from late February to late April.

Oestradiol test

Blood samples were collected from all birds in Groups 1–3 on day 0, 6, 18, 27, 38, 47, 61, and 68 of the experiment between 9 and 12 a.m. About 300 µl of blood was drawn from the jugular vein, utilising a heparinized syringe, and centrifuged within one hour. The plasma was stored at –20°C until analysed.

In the free-living birds (Group 4), blood was collected as soon as possible after capture, normally within 10 minutes. These blood samples were kept on ice until centrifuged. Plasma levels of oestradiol were determined by a radioimmunoassay technique, described by Wingfield & Farner (1975).

Gonadal growth

On day 0, 14, 31, 53, and 68 after the start of the experiment, follicle growth in females of Groups 1–3 was studied by repeated laparotomies. Each bird was anaesthetised with metophane (Pitman Moore). A slit was made in the left side between the two lower ribs. The ovary was held with blunt instruments and measured under a dissection microscope. Due to the fast and powerful growth of the ovary, it was impossible to measure the total length of this organ. Instead, the largest follicle was measured to the nearest 0.2 mm. Testes of males from Group 1 were also inspected and measured following the same procedure. Immediately after blood collection, the free-living birds of Group 4 were anaesthetised with metophane, until death occurred.

Permission has been given by the ethical committee of animal experiments Dnr. 182/93. The statistical method used was the Mann & Whitney U-test.

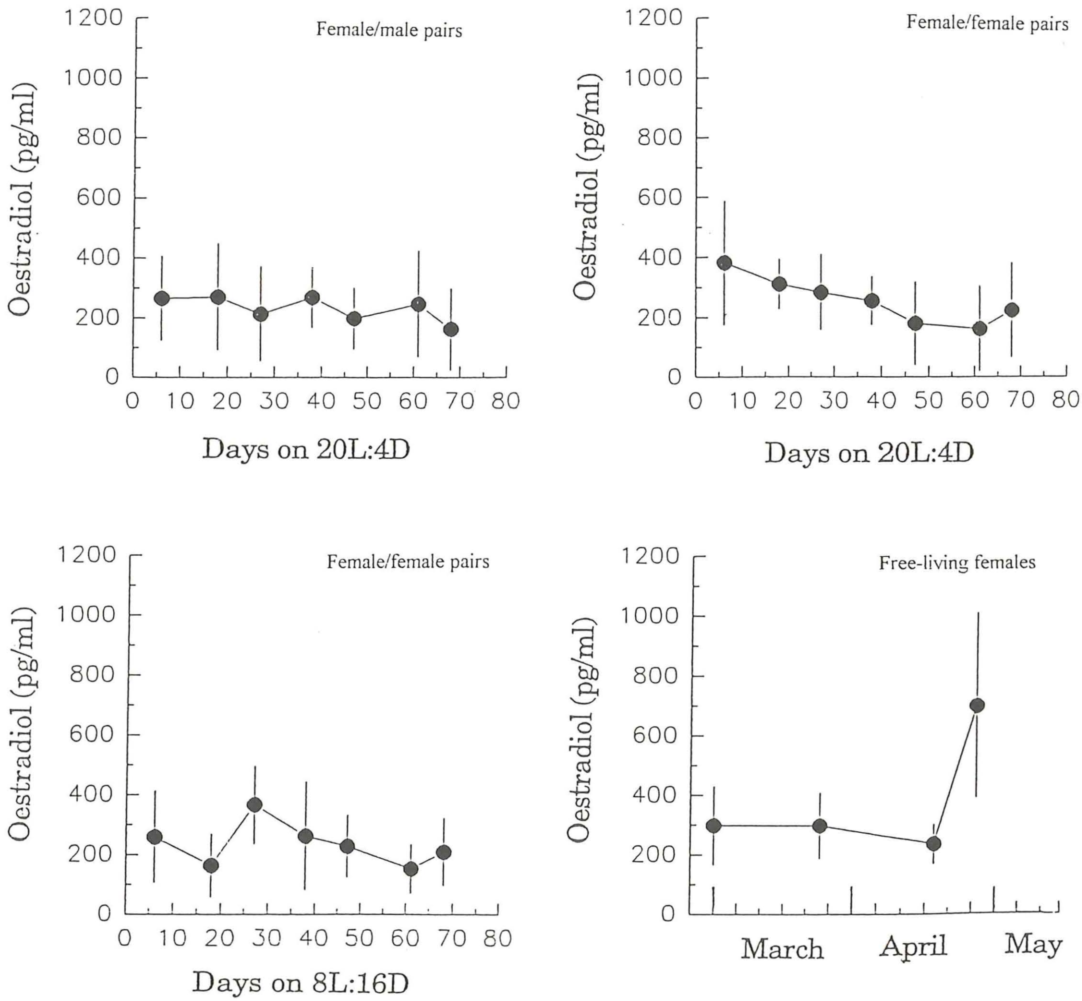

Fig. 1, a–d. Plasma levels of oestradiol in different experimental groups of female Great Tit. Values are given as means and standard deviations. Day 0=26 February.

Plasmakoncentration av östradiol hos olika experimentgrupper av talgoxhonor. Värdena är angivna som medelvärden med standardavvikelser. Dag 0=26 februari.

Results

Oestradiol

Data on plasma levels of oestradiol in the females are given in Fig. 1. Only one significant difference was noted: in free-living females, circulating levels of oestradiol increased between the nest-building and the egg-laying periods ($p<0.05$).

All females of Groups 1–3 showed the same basic level of oestradiol throughout. This level was significantly lower than the peak value of the free-living birds ($p<0.05$).

Follicle size in free-living birds

Data concerning the largest follicle are shown in Table 1. The follicle growth did not start until after two months, its size eventually increased almost tenfold ($p<0.05$).

Follicle and testis size in experimental birds

The sizes of the largest follicle and testis in the experimental birds (Groups 1–3) are shown in Table 2. The follicle of both Group 1 and Group 2 females

Table 1. Diameter of the largest ovarian follicle in free-living females at different times of the study period (Group 4). Values are given as means and standard deviations.

Diametern av den största follikeln i äggstocken hos frilevande talgoxhonor fångade vid olika tillfällen under studieperioden (Grupp 4). Värdena är angivna som medelvärden med standardavvikelser.

	N	Largest follicle size *Största follikelstorlek* (mm)
February 26	4	0.6±0.1
March 26	4	0.7±0.0
April 19 Nestbuild./*Bobygg.*	4	1.7±0.6
April 28 Egglay./*Ägglägg.*	4	5.9±2.6

peaked during the study ($p<0.01$ in both cases), those of Group 1 considerably earlier than Group 2, indicating a time difference of a couple of weeks, or more. However, the maximum follicular size measured in Group 1 females was significantly smaller than among Group 2 females ($p<0.05$).

The maximum size of the two groups was significantly smaller than that of the free-living females ($p<0.02$). Females in the control group (Group 3) did not show any significant increase in follicle size during the experiment.

Testicular size increased significantly from day 1 until at least day 31 ($p<0.05$). By day 68, the size of the testes had decreased significantly ($p<0.05$).

Nest-building behaviour

Only the females of Group 1 were provided with nest-building material. Except for one pair there were no signs of breeding activities (nest-building).

Discussion

To obtain full ovarian maturity, the female needs supplementary stimuli other than increasing day length. However, full maturation seldom happens in captivity (Farner 1964, Silverin et al. 1989, Westin 1989). This experiment was performed in order to study whether the presence of a male, a nest-box and nest-building material could stimulate the female reproductive system to accomplish full maturation. The results showed that, under the prevailing laboratory condition, this was not the case.

The stimuli, other than increasing daylength, are considered to synchronise the gonadal and hormone cycles of the female and the male in order to optimize the breeding conditions (Lehrman 1961, Hinde 1965, Lewis & Orcutt 1971, Immelmann 1973, Murton & Westwood 1977, Silver 1978, Wingfield & Farner 1980). Such stimuli usually consist of cues related to male courtship behaviour e.g. song, partner feeding or visual display (e.g. Ficken 1960,

Table 2. Diameter of the largest ovarian follicle (females) and length of the left testis (males) for Great Tits in Groups 1–3. Values are given as means and standard deviations. Group 1: female/male pairs (20L:4D), Group 2: female/female pairs (20L:4D), Group 3: female/female pairs (8L:16D) as a control group. Variation in N-values within groups is due to different measuring success.

Diametern av den största follikeln i äggstocken (honor) och storleken av den vänstra testikeln (hanar), hos talgoxar i Grupp 1–3. Värdena är angivna som medelvärden med standardavvikelser. Grupp 1: honor och hanar parvis (20L:4D), Grupp 2: honor parvis (20L:4D) och Grupp 3: honor parvis (8L:16D) som kontrollgrupp. Variation i N-värde inom grupperna beror på variation i mätframgång.

Day *Dag*	N	Group 1 follicle size *follikelstorlek* (mm)	N	Group 1 testicular size *testikelstorlek* (mm)	N	Group 2 follicle size *follikelstorlek* (mm)	N	Group 3 follicle size *follikelstorlek* (mm)
0	4	0.6±0.1	4	1.6±0.1	4	0.6±0.1	4	0.6±0.1
14	5	1.0±0.3	3	4.5±0.3	6	1.4±0.4	3	0.4±0.1
31	7	1.4±0.5	3	6.7±0.6	6	1.6±0.3	4	0.4±0.4
53	7	1.0±0.4	6	5.6±1.1	7	1.8±0.4	4	0.5±0.1
68	3	0.8±0.3	3	3.3±0.4	3	1.2±0.2	3	0.7±0.1

Brockway 1965, Cheng et al. 1988). During the spring it is the male who initiates the breeding season by securing and advertising his breeding territory, leaving to the female to make the final decision when to start the egg-laying (Farner & King 1973).

In the present experiment the oestradiol levels in females from all groups remained low, and not significantly different, throughout the experimental period. This low level was the same as that observed in free-living females prior to the nest-building period. Thus, when comparing Group 1 and Group 2 females kept on long days, it was obvious that the company of a male affected neither the oestradiol secretion, nor the follicular growth. As the testes in the males kept on long days reached full maturity, and as the testosterone level is known to increase dramatically in males kept on long days (Silverin, unpublished data) there could be no satisfactory explanation as to why the sexually mature males did not promote the maturation process in the females in this experiment. It might be that an unkown environmental factor was missing, or that the gonadotrophin secretion in the females was depressed by stress (i.e. increased corticosterone).

In free-living females, the circulating concentrations of oestradiol increased significantly during the egg-laying period. The low plasma level of oestradiol during the nest-building period is difficult to explain, because the oestradiol dependent synthesis of yolk, necessary for follicle maturation, is then in full progress (Knight & Schechtman 1954, Schejeide 1963). The breeding status of the free-living females was carefully investigated by repeated inspections of the actual nest-boxes.

On the other hand, in female Willow tits *Parus montanus*, plasma levels of oestradiol increased significantly between March and April, parallel to an increase in the LH secretion. In this species, the oestradiol levels reached a maximum during nest-building, decreasing during the egg-laying period (Westin 1989).

In this experiment, females kept on short days (8L:16D) did not show any increase in the size of the largest ovarian follicle, whereas a long day regimen (20L:4D) induced incomplete ovarium and follicle growth (Table 2). The maximum follicle size of the females kept on long day was about the same as that of the free-living females in the early nest-building period, after which the ovaries normally increase dramatically in size. This follicle growth in free-living females occurred concomitant with an increase in circulating levels of oestradiol, and it is likely that this is a prerequisite for the follicular growth. It is possible, therefore, that the retarded ovarian growth in the captive females was due to insufficient oestradiol production. Surprisingly enough, the presence of a male obviously had no effect on the latter.

Under natural conditions, slowly increasing day length initiates the photoperiodic stimulation. In contrast, the birds kept long days were instantly hyperstimulated (day length 20 hours), which enhanced the slow growth of the gonads. Examination of the long day stimulated pairs of Group 1 showed that the gonads of both sexes synchronously increased and regressed in size. This regression of the follicles started just before their transformation into "preovulatory follicles". The reason for this could be that the males, being sexually weaker as a consequence of the regressing testes, failed to stimulate the female properly at a critical point of follicle development. This could possibly be tested by replacing the "regressive" males with males in an earlier phase of the sexual cycle.

Comparing the females kept on long days, the size of the largest follicle in Group 1 was significantly smaller throughout than that of Group 2, where the growth was faster and the largest follicle peaked later (Table 2). At least two explanations of this difference are conceivable. 1: The males of Group 1 generally inhibit the maturation process. 2: The females of Group 2 stimulate each other as competitors awaiting the appearance of a male, on the supposition that he will choose the most mature (= attractive) one of the two. Obviously, this kind of stimulation does not end up in complete maturity. Unfortunately, the exact breeding status at the peak of the ovarian development is not known as these females had no access to nest-building material.

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Sammanfattning

Hur påverkas talgoxhonans Parus major *könsmognad av ökande dagslängd och närvaro av hane?*

Talgoxhonans svar på den ökande dagslängden undersöktes, vad gäller äggstockarnas tillväxt, både i fält och på laboratorium. På våren när dagslängden passerat 11 timmar startar ljuset talgoxens könsmognadsprocess. Hos fåglar förmedlas den fotoperiodiska informationen genom att ljuset tränger igenom skalltaket och hjärnvävnaden, där det träffar ljuskänsliga celler. Dessa stimulerar hormonproducerande nervceller i hjärnan. Observera att ögonen inte har någon funktion i detta sammanhang. De stimulerade hormonella processerna påverkar både tillväxten och hormonproduktionen hos könsorganen. Hos honan bildas könshormonet östradiol av särskilda celler i äggstocken. Östradiol påverkar en rad nödvändiga processer under häckningsperioden, bl.a. gule-syntesen i levern, ruvfläckens bildning och beteendemönstrets utveckling hos honan.

Talgoxarna påverkas även av andra faktorer som påskyndar eller bromsar könsmognaden. Exempel på dessa är tillgången på föda, närvaro av en uppvaktande hane, boholk och andra miljöfaktorer, vilka alla till skillnad från dagslängden uppfattas direkt av ögon och öron.

Talgoxhonans äggstock tillväxer vid stimulering både i storlek och vikt. Den sexuellt aktiva hanens beteende gentemot honan är viktig för att stimulera denna process och för att samordna honans fortplantningscykel med den egna. Detta sker hos talgoxen främst från början av bobyggnads-perioden och under äggläggningsperioden. Hanen behöver utöver ökningen i dagslängden ingen stimulering från honan för att få igång spermieproduktionen.

Syftet med detta experiment var att utröna om könsmognaden hos talgoxhonan går fortare och blir fullständig vid närvaro av en sexuellt aktiv hane, holk och bobyggnadsmaterial.

Metoder

I februari sattes två grupper med talgoxhonor i rum där ljuset var tänt 20 timmar och släckt 4 timmar varje dygn (20L:4D). Den ena gruppen bestod av 8 burar med en hona och en hane tillsammans med boholk och bomaterial i var och en (Grupp 1), och i den andra fanns 4 burar med endast två honor i varje (Grupp 2). En tredje grupp av honor hölls under kortdags förhållanden (8L:16D) och utgjorde en kontrollgrupp om 4 burar med två i varje (Grupp 3). Dessutom infångades frilevande honor under perioden februari-april (Grupp 4).

Blodprover togs på honorna med olika tidsmellanrum för att följa hur könshormonet östradiol varierade under studien. Äggstockens tillväxt undersöktes genom att snitta ett hål i kroppsväggen och med hjälp av ett mikroskop mäta diametern på den största äggfollikeln.

Resultat

Blodets innehåll av östradiol visade hos de frilevande honorna en signifikant ökning som började under bobyggnadsperioden och nådde högsta värdet under äggläggningperioden (Fig.1). Honorna i bur visade samma låga nivå hela tiden (Fig. 1).

Äggfollikelstorleken ökade kraftigt under bobyggnads- och äggläggningperioden hos de frilevande honorna (Tabell 1), medan folliklarna hos de honor, som hölls i bur, ökade endast till samma storlek som hos de frilevande honorna under bobyggnadsperioden för att därefter tillbakabildas igen. Egendomligt nog var follikeltillväxten hos honorna i Grupp 2 snabbare och kraftigare än hos honorna i Grupp 1 (Tabell 2).

Diskussion

Syftet med experimentet var att undersöka om könsmognadsprocessen hos talgoxhonorna blev förstärkt och kanske fullständig genom närvaron av en hane, boholk och bomaterial. Resultaten visade ingen sådan påverkan, trots att enligt flera forskare en sådan extra stimulering från bl.a. hanen är nödvändig för att honan skall nå äggläggningsstadiet. Inte heller visade talgoxhonorna någon skillnad mellan Grupperna 1-3 vad beträffar östradiolproduktionen. Alla låg hela tiden på samma låga nivå som de frilevande honorna vid bobyggnadsperiodens början. Att närvaro av en hane inte haft någon effekt kan möjligen tillskrivas någon stressfaktor som orsakats av de experimentella omständigheterna.

Studien visade att kortdagshonorna (Grupp 3) inte fick någon follikeltillväxt. Hos långdagshonorna (Grupp 1–2) inträffade en ofullständig follikel- och ovarietillväxt. Honorna i Grupp 2 visade dock en något senare inträdande och markant större follikelstorlek. Kanske kan detta tolkas som att honorna stimulerade varandra som konkurrenter i väntan på en hane, och då under förutsättningen att den hona, som kommit längst i könsmognadsförloppet, har störst chans att vinna en uppdykande hanes gunst.

Normalt under våren stimuleras könsmognaden av en långsamt ökande dagslängd. I denna studie "chockstimulerades" hanarna genom att de överfördes från kortdag utan övergång till långdag. Detta påskyndade hanens testikeltillväxt. Testiklarna började sedan tidigt att tillbakabildas, redan långt innan honornas äggstockar hunnit att utvecklas färdigt. Kanske är en hane med krympande testiklar inte i det tillstånd som krävs för att effektivt stimulera honans mognadsförlopp?

Eftersom östradiolhalten steg kraftigt under äggläggningsperioden hos de frilevande talgoxhonorna, är det troligt att ökande halter östradiol krävs för att äggstockens folliklar skall kunna tillväxa (under inlagring av gula) just innan äggläggningsperioden.