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AN ARTIFICIAL SIMULATION OF THE VERNAL INCREASE IN DAY LENGTH AND ITS EFFECTS ON THE REPRODUCTIVE SYSTEM IN THREE SPECIES OF TITS (*PARUS* SPP.), AND MODIFYING EFFECTS OF ENVIRONMENTAL FACTORS–A FIELD EXPERIMENT[1]

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Abstract. Analyses of breeding data from a coniferous forest in southwestern Sweden showed that Crested Tits (*Parus cristatus*) laid their first eggs about 2 weeks earlier, at a time when day lengths were about 1.5 hr shorter, than did Willow Tits (*P. montanus*) and Great Tits (*P. major*). In a laboratory study we investigated whether this difference was correlated with differences in gonadal growth and circulating levels of LH induced by increasing day length. It was found that the rapid testicular growth phase, in all three species, started when day length exceeded 12 hr. However, in Crested Tits and Great Tits plasma levels of LH started to increase when day length reached 11 hr, and in Willow Tits, 14 hr.

This last result prompted a field experiment where we investigated how testicular growth and LH and testosterone titers in Willow Tits were affected by environmental factors such as population density and/or food availability during late winter to early spring. Samples were collected in mid-February and mid-March. Results showed that LH and testosterone secretion in males from a population given extra food during the entire winter, and as a consequence of this having a high population density, increased much earlier than the laboratory results predicted. However, in accordance with our laboratory data, LH levels in females from the manipulated population, and in males and females from the control population (given no extra food during the winter), did not increase between February and March. It is speculated that the endocrine effects on the "experimental males" were caused by an increased density of the Willow Tit population, and its effects on territorial behavior. The results are also discussed in comparison with earlier studies on free-living Great Tits and Willow Tits. It is concluded that, despite our laboratory data, plasma LH concentrations in Willow Tits can increase at the same time as in Great Tits and Crested Tits, but that evolution has come to utilize this physiological capacity differently when determining the breeding times for Crested Tits, Willow Tits, and Great Tits.

Key words: Tits; Parus; *photoperiodism; gonads; LH; steroids; population density; territorial behavior.*

INTRODUCTION

In southwestern Sweden, Great Tits (*Parus major*), Willow Tits (*P. montanus*), and Crested Tits (*P. cristatus*) are common forest-dwelling species with somewhat different habitat preferences. Great Tits are found in both deciduous and coniferous forests (more numerously in the former habitat), whereas Willow Tits breed in mixed and coniferous forests. Crested Tits are found only in coniferous forests. Both Willow Tits and Crested Tits are territorial outside the breeding period. In recent years we have performed a number of photoperiodic experiments on all three species. The results show that all are truly photoperiodic (Silverin, unpubl. data; Viebke and Westin, unpubl. data). As all species commonly breed in nest boxes their breeding ecology is well documented, especially that of the Great Tit (e.g., Gibb 1950, von Brömsen and Jansson 1980, Källander 1983, Ojanen and Orell 1985). For a number of years the breeding biology of the three species has been carefully followed in study areas east of Gothenburg. Results from these studies revealed that Crested Tits laid their first eggs about 2 weeks earlier than did Great Tits and Willow Tits.

In the present study we primarily wanted to investigate whether this temporal difference in the onset of egg laying (which corresponds to a difference in day length of 1.5 hr) was related to

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species differences in photoinduced increase of plasma levels of LH and testicular development. In other words, will the reproductive system of the Crested Tit be activated earlier, i.e., by shorter day length, than that of Willow Tits and Great Tits? If this is the case, it could explain why Crested Tits start to breed earlier than the other species. Or do all species show the same pattern of LH titers and testicular growth?

The results from these studies led us to perform a field experiment, where we tried to modify, in free-living Willow Tits, the time when LH titers started to increase and when the onset of fast gonadal growth occurred. The results obtained from captive birds were compared with those from free-living birds.

MATERIALS AND METHODS

Birds were captured in November with the help of a tape recorder and mist nets. The sex of Willow Tits and Crested Tits was determined by laparotomy and males were kept two per cage in temperature- and light-regulated rooms. Birds were kept on a natural day length (L:D, 8:16) for 2 weeks before the experiment started. The experiment lasted for 14 weeks. Males of each species were divided into four groups ($n = 8$ for Great Tits and Willow Tits, and $n = 6$ for Crested Tits). Two groups were used for blood sampling, and two for laparotomy. Temperature was kept constant throughout the experiment (+18°C), but "day length" (hours of light) was increased by 30 min each week ("sunrise" 15 min earlier, and "sunset" 15 min later). Each day was preceded and followed by 5 min of dim light. Birds were supplied with water, vitamins, and food ad libitum (sunflower seeds, dried insects, live maggots, and mealworms). On the last day of each photoregime, about 150 μl of blood was collected from the jugular vein into a heparinized syringe. After centrifugation, plasma was stored at −20°C until analyzed. As we had two groups of birds for blood sampling, blood was collected from each individual at 2-week intervals. Laparotomies were performed every other week and on the last day of a weekly photoregime. As there were also two groups of birds from each species for this purpose, each individual was laparotomized every fourth week.

To estimate when free-living tits began their sexual activities and established breeding territories ("singing birds") we analyzed bird census data from the years 1968–1978. During these years, birds were counted by six ornithologists (among others B. Silverin) during the first weekend of each month (using the line-transect method) along a 20-km route through our study areas, a coniferous forest east of Gothenburg, southwestern Sweden.

The results from the photoperiodic studies prompted a field experiment where we investigated how testes growth and LH titers in Willow Tits were affected by environmental factors such as population density and/or food availability in March, when day length was approximately 12 hr. By providing extra food continuously during the entire winter, winter survival was greatly improved among Willow Tits (Jansson et al. 1981). During one autumn/winter, tits within a 1.2-km^2 area were fed sunflower seeds from 19 automatic feeders. Feeders were regularly refilled with seeds throughout the winter. Birds visiting the feeders were captured and color-banded during November and December. The area was visited regularly during the winter, and the movements of the tits were followed. In mid-February, i.e., before the rapid testicular growth phase started, and in mid-March a number of birds that had fed at the feeders throughout the winter were captured. Control birds were captured in a coniferous forest, about 20 km east of the fed population, where no supplementary feeding had occurred. The exact time of netting was recorded for each bird. Thus, at the time the blood sample was collected, we knew exactly how many minutes (between 2 and 10 min) the bird had been trapped. Blood was collected as described above. In the field, syringes were kept on ice. Plasma was separated and frozen later the same day. After blood sampling, the bird was decapitated, the organs fixed in Bouin's fluid, which was replaced by 70% ethanol within 48 hr. At the laboratory, testes were weighed to the nearest 0.05 mg on a Mettler precision balance after having dried for 30 sec on a paper towel.

Plasma levels of LH were measured by the double antibody radioimmunoassay technique of Follett et al. (1972), as modified by Follett et al. (1975). Steroids were assayed after separation on Celite : glycol microcolumns (Silverin et al. 1984, 1986).

For the laboratory experiment the Wilcoxon's matched-pairs signed ranks test was used for statistical analyses. In the other studies a two-tailed Mann-Whitney *U*-test was used.

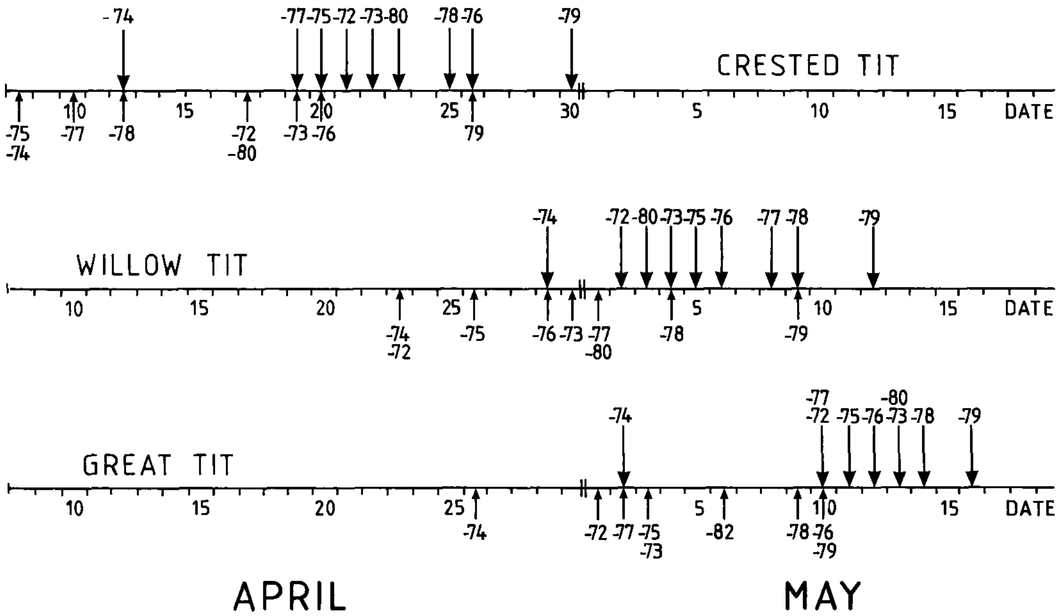

FIGURE 1. The onset of egg laying for three species of tits in a coniferous forest in southwestern Sweden during the years 1972–1980. The arrows below the calendar-axis give the date for the first egg laid within the population. The arrows above the calendar-axis show the average time of the population for laying of the first egg in a clutch.

RESULTS

BREEDING DATA

Figure 1 summarizes data regarding onset of breeding (defined here as the laying of the first egg in the clutch) during the years 1972–1980 in our study areas. The mean date for the first egg laid by any female within the population and the mean date for the first egg for all breeding pairs were: Crested Tit, 15 April and 22 April, respectively; Willow Tit, 30 April and 6 May, respectively; Great Tit, 4 May and 13 May, respectively.

SINGING BIRDS

There were no significant differences within species between early December and early February in the proportion of observed birds that were singing (Fig. 2). The proportion increased significantly between February and March in Great Tits and Willow Tits ($P < 0.02$ in both cases), but not in Crested Tits. For the latter species there was however a significant increase between January and March ($P < 0.002$). In early March significantly more Great Tits than Willow Tits and Crested Tits were observed singing ($P < 0.004$). Although the proportion of singing birds increased significantly between March and April for all species ($P < 0.05$), there was no longer any significant difference between Great Tits and Willow Tits, but there was still such a difference between Great Tits and Crested Tits ($P < 0.002$). After April the proportion of singing birds increased significantly for Willow Tits ($P < 0.002$) and Crested Tits ($P < 0.05$), but not for Great Tits. In early May significantly more of the observed Great Tits and Willow Tits were singing than was the case for the observed Crested Tits ($P < 0.002$).

PHOTOPERIODIC EXPERIMENT

Results are summarized in Figures 3A, 3B, and 3C. For the Crested Tit there was a significant difference in testicular size between birds exposed to 10 and 11 hr of light (L) ($P < 0.02$). Testes continued to increase significantly in size between each laparotomy ($P < 0.05$ in each case), except between the last two occasions when there was instead a significant decrease ($P < 0.05$). Plasma levels of LH increased drastically between birds exposed to 10.5L and those exposed to 11L ($P < 0.02$). At longer photoregimes LH concentrations remained consistently high.

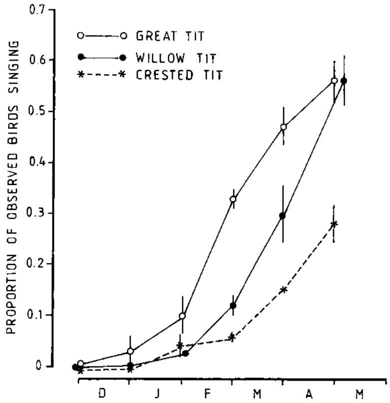

FIGURE 2. The proportion of observed birds that were singing at different times of the season. Data originate from bird censuses (line transect method) in a coniferous forest in southwestern Sweden during the years 1968–1978. Figures are given as $\bar{x} \pm$ SE.

For the Willow Tit there was a slight, but significant, increase in testis size between 8L and 11L ($P < 0.02$). Testis length is significantly greater in males exposed to 13L than in males exposed to 12L ($P < 0.02$). Growth continued in birds exposed to 14L ($P < 0.02$). After this time testes did not grow any further. Although there were small, but inconsistent, fluctuations (some groups even significantly different from each other) in plasma levels of LH in birds exposed to photoperiods between 8L and 13.5L, the sudden and drastic increase in LH titer occurred when Willow Tits were exposed to 14L ($P < 0.02$). LH levels remained high in birds exposed to 14.5L and 15L.

In Great Tits, testes increased slightly, but significantly, in size between birds exposed to 8L and those exposed to a day length of 10 hr ($P < 0.05$). The slow, but still significant, increase in testis size continued between 10L and 11L ($P < 0.05$). After having reached a day length of 12 hr a more rapid growth started, and there was a significant increase in testis size between birds exposed to 12L and 13L ($P < 0.05$). There was no significant difference in testis size between Great Tits exposed to 14L and 15L. Plasma levels of LH increased significantly after exposure to 11 hr of light ($P < 0.02$). LH titers continued to increase significantly during the following weeks ($P < 0.02$; light regimes 11.5L : 12.5D and 12L : 12D). A transitory peak was reached in birds exposed to 12L. Thereafter, plasma levels of LH decreased significantly ($P < 0.02$) to more mod-

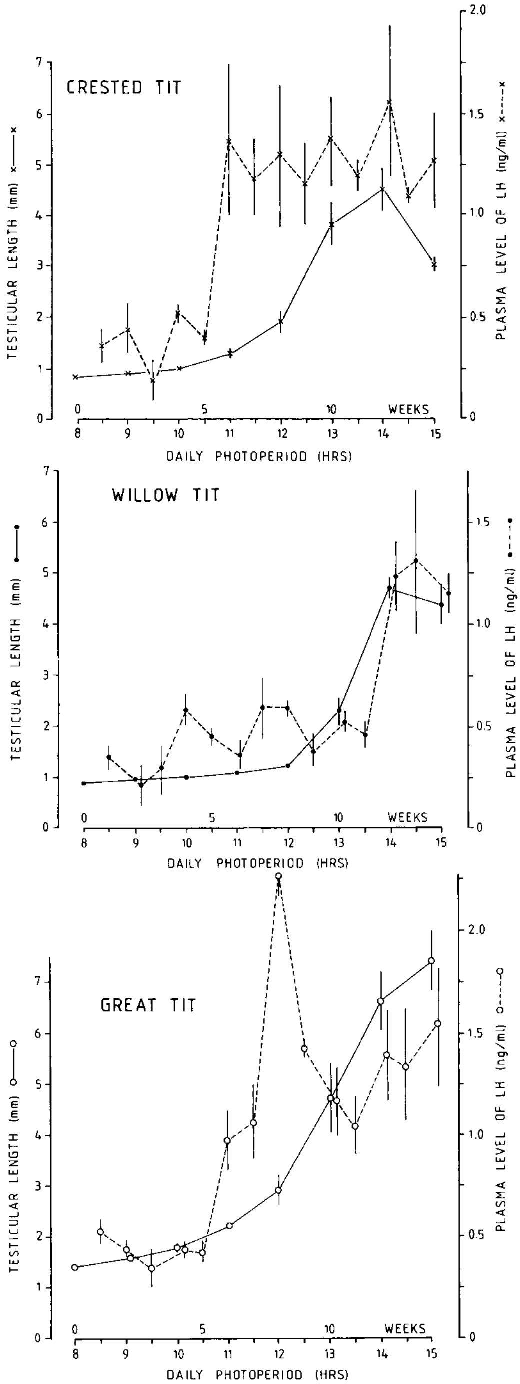

FIGURE 3. Results from the photoperiodic experiment simulating the increasing day lengths in spring, and its effect on gonadal growth and circulating levels of LH for three species of tits. At the beginning of the experiment the light : dark cycle was 8:16 (hr). The light period was increased 0.5 hr per week. Figures are given as $\bar{x} \pm$ SE.

TABLE 1. Plasma levels of some hormones and gonadal size in Willow Tits from a fed (experimental) population compared with those from Willow Tits in an undisturbed (control) population in mid-February and mid-March. DHT = dihydrotestosterone; T = testosterone; C = corticosterone. All figures are given as $\bar{x}$ = SE.

	February		March	
	Male	Female	Male	Female
		Experimental population		
LH (ng/ml)	0.91 ± 0.19	0.64 ± 0.16	2.06 ± 0.12	0.65 ± 0.05
DHT (pg/ml)	100.1 ± 13.7	79.5 ± 4.7	150.1 ± 4.8	67.6 ± 3.8
T (pg/ml)	198.0 ± 39.1	74.2 ± 4.4	849.0 ± 96.8	79.4 ± 5.4
C (ng/ml)	14.3 ± 0.1	16.6 ± 3.4	19.6 ± 3.0	8.3 ± 2.0
Gonad (mg)	1.9 ± 0.0	4.2 ± 0.6	6.6 ± 0.9	5.0 ± 0.5
n	7	10	10	8
		Control population		
LH (ng/ml)	1.08 ± 0.32	0.47 ± 0.06	1.17 ± 0.25	0.71 ± 0.11
DHT (pg/ml)	83.8 ± 3.9	65.4 ± 1.1	158.7 ± 21.2	71.6 ± 5.5
T (pg/ml)	135.0 ± 36.2	105.6 ± 26.7	459.4 ± 121.8	165.7 ± 64.4
C (ng/ml)	10.8 ± 1.1	23.4 ± 8.0	10.5 ± 1.5	7.9 ± 1.4
Gonad (mg)	1.0 ± 0.1	1.4 ± 0.2	6.7 ± 0.9	4.5 ± 0.4
n	10	10	9	8

erate levels. These levels were however still significantly higher than LH levels in birds exposed to 10.5L or less ($P < 0.02$ in all cases).

FIELD EXPERIMENT

Results are summarized in Table 1. Out of the 19 feeders, 13 were utilized by mixed groups of tits throughout the winter. The groups mainly consisted of Willow Tits, Crested Tits, and Coal Tits (*P. ater*).

Details of the population dynamics in the fed population are beyond the scope of this paper. However, in summary it can be stated that the birds moved quite freely between feeding stations, and territorial borders were not as strict as could be expected.

To estimate winter group size in the two populations a number of Willow Tit groups were followed more carefully in mid-February. Group size in the control population was 1.5 ± 0.1 (n = 10), and in the experimental population 3.0 ± 0.4 (n = 9).

In the control population LH levels did not increase significantly, in either sex, between mid-February and mid-March. In the fed population LH levels increased significantly during this period in males ($P < 0.002$), but not in females. In February there were no differences in LH levels between males from the two populations, nor between females. In March males, but not females, from the fed population had significantly higher plasma levels of LH than unfed males ($P < 0.05$).

At this time of the year (February and March) we found no correlations between plasma levels of any hormone and the time (between 2 and 10 min) the bird had spent in the mist net. Female Willow Tits showed no variations in dihydrotestosterone, testosterone, or corticosterone titers between any group or period. In both populations plasma levels of dihydrotestosterone and testosterone increased significantly in males between mid-February and mid-March (DHT $P < 0.05$ in both cases; T $P < 0.002$ in both cases). In February, males from the two populations had similar testosterone levels, but in March fed males had increased their testosterone levels significantly more than males in the control population ($P < 0.05$). Males, but not females, from the fed population had significantly higher plasma levels of corticosterone than males from the control population in both February and March ($P < 0.05$ in both cases).

In the control population, gonads, in both sexes, grew significantly between mid-February and mid-March ($P < 0.002$ in both cases). In the fed population ovaries did not increase in size during this period, but testes did ($P < 0.002$). Gonads from males and females in the fed population were significantly heavier than those from birds in the control population in February ($P < 0.002$ in both cases), whereas there were no differences between the two populations, in either sex, in March.

DISCUSSION

GONADAL GROWTH AND TIME OF EGG LAYING

Tits are seasonal breeders that maintain functional gonads only during a few spring and summer months. Seasonality in northern latitudes normally involves a time-measuring system that informs the bird about the approaching breeding season. This information must reach the bird well before optimal breeding conditions are at hand. Birds in the north use changes in the day length to control reproductive development, and when exposed to long day periods gonads will grow as a result of an increased secretion of gonadotrophins (reviews e.g., Murton and Westwood 1977, Wingfield and Farner 1980, Follett and Robinson 1980, Farner 1985).

Crested Tits start egg laying about 2 weeks earlier (mid-April) than Willow Tits and Great Tits. In our experimental simulation of increasing day lengths in spring, testes from all three species entered a "rapid growth phase" when the birds were transferred to day lengths between 12 and 13 hr. Twelve hours of daylight corresponds to natural day lengths in mid-March, and 13 hr of daylight corresponds to early April. Thus, differences in photoinduced testicular development cannot explain the observed differences in the time of onset of egg laying. Our laboratory data correspond well with data on free-living Great Tits (see Fig. 4) and Willow Tits (Viebke and Westin, unpubl. data). Data for Crested Tits are lacking.

Females were not included in our laboratory experiment. When it comes to the question on the time of egg laying, Perrins (1970) argues that female Great Tits will produce a clutch as soon as the food supply in spring permits. In fact there are several studies showing a relationship between mean hatching date of Great Tits and the date of maximum food availability (e.g., van Balen 1973). Experimental studies on Great Tits, Willow Tits, and Crested Tits have shown that the onset of egg laying can be advanced by providing extra food (von Brömsen and Jansson 1980, Källander 1983). However, egg laying was only advanced by 3 to 7 days, and hence was not enough to correspond to the time when males

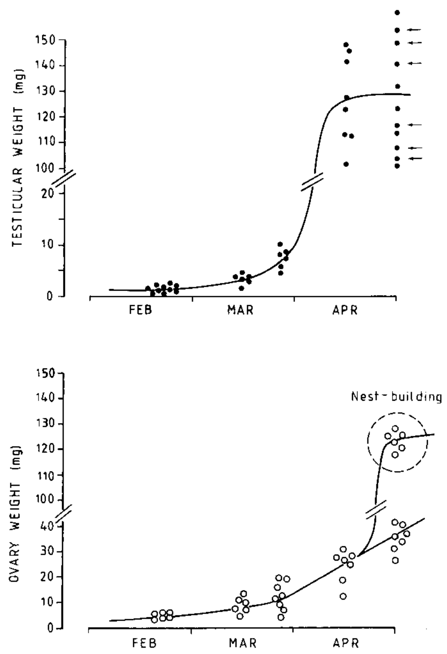

FIGURE 4. Gonadal size of Great Tits in relation to season. The testes in free-living males reach full size already in early/mid-April, i.e., well before egg laying starts. Arrows show males paired with a nest-building female. Female Great Tits need other stimuli, besides photoperiodic stimuli, to induce final maturation of ovarian follicles. Data from free-living Great Tits collected in 1973 by B. Silverin. Arrows show data for males paired with a nest-building female.

attained maximum testicular size, i.e., mid-April. Our field experiment showed that female Willow Tits were not at all affected by the increased food availability and/or population density in the way males were. This might be due to the quality of the food, or to low air temperatures. In mid-March, snow was still covering the ground in our study area, and air temperature, or its effects on the food supply, is known to be of vital importance for annual variations in the onset of egg laying in Great Tits (e.g., Kluyver 1952, Slagsvold 1976). Although laying starts at much lower temperatures in Crested Tits than in Willow Tits and Great Tits, the start of laying in Crested Tits is also known to be affected by small changes in the ambient temperature (Ojanen and Orell 1985). In April, when Crested Tits start egg laying, the daily mean temperature is only a few degrees above zero, and the food supply has probably not increased very much after winter.

As also illustrated in Figure 4, photostimuli cause only partial development of the ovary in free-living Great Tits in March and April. Maximum follicular development does not occur until nest building has started. The increase in female LH secretion follows the same pattern! Thus, nonphotoperiodic sources of information play a different role in the final development of gonads in females than in males, and it is quite obvious that the female determines when egg laying is to start, and the male, although fully prepared for breeding, just has to wait for the female to be prepared. This is in agreement with most other studies (reviews Lofts and Murton 1968, Farner and Lewis 1971).

GONADAL WINTER GROWTH

In the laboratory, Crested Tits and Great Tits, and to a lesser extent Willow Tits, also showed testicular growth, although at a slower rate, on photoperiods less than 12 hr, i.e., light regimes that we normally consider nonstimulatory. Unfortunately we do not have any data on Crested Tits, but in free-living Great Tits (Silverin, unpubl. data) and Willow Tits (Viebke and Westin, unpubl. data) both sexes show a similar slow but significant gonadal growth that starts in December/January. This growth is more pronounced in adults than in juvenile birds. During late autumn (October to December) there is no increase in gonadal weight in any sex or age group. Although it is known that a reactivation of the steroidogenic ability of gonads occurs immediately after breaking of refractoriness in several bird species (e.g., Nicholls et al. 1988), the existence of a slow gonadal winter growth starting 3–4 months after breaking of refractoriness, and when day lengths are well below stimulatory length, is generally not recognized. On the contrary, it is usually stated that gonadal growth does not occur until the critical photoperiodic threshold is reached. However, some authors have observed a similar, so-called nonphotoperiodic growth. Farner et al. (1966) point out the existence of a linear ovarian growth in first-year White-Crowned Sparrows (*Zonotrichia leucophrys gambelii*) during the autumn/winter period that, according to the authors, must be independent of the daily photoperiod. Also in Japanese Quail (*Coturnix coturnix*) (Follett and Maung 1978) and Tree Sparrows (*Spizella arborea*) (Wilson and Follett 1974) testes grow slowly if the birds are kept on photoperiods shorter than the critical day length.

At present, we do not know what initiates this slow growth in tits in December/January. In free-living birds the onset of the slow gonadal growth phase is concomitant with the significant and lasting increase in LH secretion (no increase in plasma levels of testosterone occurs at this time) in both sexes of Great Tits and Willow Tits (Röhss and Silverin 1983; Silverin et al. 1986; Silverin, unpubl. data). Also, if photorefractory Great Tits, captured in August, are maintained on short days (8:16) for a year, testes start to grow and plasma levels of LH increase in early March (Silverin, unpubl. data). Therefore, it might be that the induction of the slow gonadal growth is caused by a seasonal change in the sensitivity to the negative feedback of testosterone (such a system exists in the Great Tit; Silverin, unpubl. data), and/or a slow increase in the testicular concentration of LH and FSH receptors. Or it might be a reflection of some endogenous rhythm and/or a consequence of the number of short days to which the birds have been exposed. Or, a controversial thought, might it be that short days not only break refractoriness, but also in some way actually turn the reproductive system on?

LH AND EARLY SPRING PHOTOREGIMES

Our LH data are difficult to interpret. A rapid increase in circulating levels of LH occurred in Crested Tits and Great Tits after exposure to 11L but not in Willow Tits until day length had reached 14 hr. Thus, in Crested Tits and Great Tits plasma levels of LH increased before the testicular rapid growth phase had started, whereas in Willow Tits it did not occur until day lengths had become much longer than those needed to induce rapid testicular growth. Why these temporal differences among species?

Comparing these laboratory results with field data from earlier studies on unmanipulated populations further complicates the picture (Röhss and Silverin 1983; Silverin et al. 1986; Silverin, unpubl. data). These field studies revealed a significant and lasting increase in LH titers in male and female Great Tits and Willow Tits already during early winter (December–January), when day lengths were less than 7 hr and temperatures ranged from 0°C to −30°C. After this first winter increase the seasonal changes in hormonal plasma levels differed between the two species. In both species LH levels remained elevated in February. In male Great Tits, plasma levels of LH increased drastically in mid-March and reached maximum levels for the year at this time, i.e., well before nesting had started but at a day length in good agreement with our laboratory observations. However, this peak in March was only transitory and was followed by a steady decline in LH levels during April and May. Also, plasma levels of LH in free-living male Willow Tits increased significantly between mid-February and mid-March, which is in contrast to our present laboratory results. Even if civil twilight is included, day length does not exceed 13 hr in mid-March. However, contrary to male Great Tits, male Willow Tits did not show any transitory March peak, but plasma levels of LH continued to increase through April and reached a maximum for the year in May—at the time of nest building. Thus, although the LH pattern of the Willow Tit was different from that of the Great Tit, the fact is that free-living male Willow Tits have the capacity to increase their LH secretion at the same time as free-living Great Tits, and thus much earlier than indicated by our laboratory results. Plasma levels of testosterone paralleled the LH curves in both species at this time of the year.

Female Great Tits also showed a drastic, significant, and consistent increase in plasma levels of LH in March, although maximum levels were not reached until nest building was almost finished. Unlike female Great Tits, free-living female Willow Tits did not show increased LH levels until mid-April, i.e., when day lengths are approaching 14 hr, and thus stand in agreement with our laboratory results on male Willow Tits.

SINGING BEHAVIOR

Contrary to our laboratory results, plasma levels of LH (and testosterone) started to increase simultaneously in free-living male Great Tits and Willow Tits (Röhss and Silverin 1983, Silverin et al. 1986). Our bird song analyses showed that all three species increased their singing activity at the same time that we observed the increased LH and testosterone levels in free-living tits. Great Tits, Willow Tits, and Crested Tits all use their song to attract a female and to proclaim a territory. It must be concluded that they all become sexually active at some time between mid-February and mid-March, i.e., at day lengths somewhere between 11–12 hr.

Great Tits within our study area leave the forest during the hard winter period, return during late winter, and establish new breeding territories in March. Willow Tits, as well as Crested Tits, remain in the forest throughout the winter where they defend large winter territories. In spring they will establish a small (when compared to the size of the winter territory) breeding territory somewhere within the borders of the winter territory. The number of surviving Willow Tits, and Crested Tits, in a winter group is on the average less than two and male Willow Tits have a higher survival rate than females (no data on Crested Tits) (Ekman 1979, pers. comm.). Thus, there is a surplus of suitable breeding territories but a lack of females in the forest. Consequently, males from these species normally do not have to compete for a breeding territory in spring, and the song of the surviving males probably mostly serves to advertise for a female. However, as unpaired, but still territorial, male Willow Tits may move over large distances (up to 7–8 km, J. Ekman, pers. comm.), probably in search of a female, the song must also serve the purpose of telling these floaters that a territory is already occupied.

Krebs (1977) showed in a study on English Great Tits that when availability of good breeding habitats is a limiting resource the song of the Great Tit mainly functions in territorial defense. In a Swedish study on Great Tits, living in a semi-open deciduous woodland, Björklund (1988) demonstrated that the Great Tit song was used to attract a female. When the Great Tits return to our study area in late February or early March they start to sing and establish new breeding territories. It is likely that in a coniferous forest (where good breeding habitats occur less numerously than in deciduous forests) the song of the Great Tit functions both in territorial defense and to attract a female.

The seasonal change in the proportion of observed singing birds may be due to the fact that males sing less frequently in March than they do in May. In late winter/early spring food is still scarce and it is likely that the males have to spend a lot of time searching for food. With the progress of spring, food becomes more readily available and the males can reduce their foraging time and instead spend more time singing.

POPULATION DENSITY, LH AND TESTOSTERONE

The discrepancy between laboratory results and field data (as is the case for the Willow Tit) can be due to lack of certain environmental stimuli in the laboratory. Survival rate and population density vary between years and thereby also territorial behavior. As both territorial and singing behaviors are intimately related to LH and testosterone secretion, it is likely that the secretion patterns will vary between years. For example, LH and testosterone levels would be expected to increase much faster in Willow Tits if population density and flock size were high at the time breeding territories are being established.

Our field experiment was designed to test this hypothesis. In a study spanning over 8 years Ekman (1988) reported that the mean group size of Willow Tit flocks was 1.35 in late winter. At the time permanent winter groups were established in late autumn, the average group size was 3.9. Thus, later winter group size in our fed population (on the average three birds per group) was much higher than normal. Furthermore, Ekman (1988) reports an average of 4.8 Willow Tit groups per km^2. Besides having larger groups we also had a much higher group density (ca. $7/km^2$) in our experimental area. These data clearly demonstrate that we had a high density of Willow Tits in the study area containing feeders.

Our studies showed that an increased food availability, during the entire winter, did not affect LH or testosterone titers in Willow Tits before they had started to establish their breeding territories. However, by mid-March i.e., when day lengths were approximately 12 hr, and singing and establishment of breeding territories had begun, plasma levels of LH and testosterone had increased significantly in males from the fed population, and were also significantly higher in these males than in those from the control population. Contrary to our earlier results (Silverin et al. 1986), but consistent with our laboratory results, males in the control population did not increase their circulating levels of LH between mid-February and mid-March. Testosterone levels were however significantly increased in both populations. That testosterone secretion and LH secretion do not always increase simultaneously has been observed on several other occasions in birds (e.g., Röhss and Silverin 1983, Wingfield 1984, Silverin et al. 1986).

There was no increase in LH levels between February and March in Willow Tit females from any of the two populations. This is consistent with our earlier results. Consequently, LH secretion patterns from both free-living female Great Tits and female Willow Tits fit well with our laboratory data. However, in only one case (our control population) out of three did male Willow Tits follow the female LH secretion pattern between February and March. In the study of Silverin et al. (1986) male Willow Tits showed a slight, but still significant, increase between these two periods. Experimental males from the present study showed a much more drastic increase between February and March.

Bad weather during early spring can inhibit gonadal development and depress testosterone secretion in birds (e.g., Marshall 1949; Lofts and Murton 1966; Wingfield 1985a, 1985b). It has also been demonstrated in a number of studies that low food availability, or a deficient diet, has dramatic antigonadal effects even under stimulatory day lengths (e.g., Assenmacher 1973; Wingfield 1980, 1983). It is notable that by supplying a population with extra food for the entire winter, the gonads were significantly larger in the fed population in February, but not in March when the rapid growth phase started. This resembles the gonadal growth pattern seen in Song Sparrows (*Melospiza melodia*) during years when early gonadal development is retarded. In these years gonadal development accelerates as spring ensues, and the gonads mature at a normal time (Wingfield 1985a). Reduced availability of food delays gonadal development in laboratory experiments. However, it does not prevent a photoperiodically induced rise in circulating LH concentrations, although testosterone levels are drastically reduced (Wingfield 1980, 1983). In our experiment, plasma levels of testosterone in males increased in both populations, and LH increased in males from the fed population. However, there were no differences in the size of the testes or ovaries between fed and unfed birds in March. These circumstances make it unlikely that the observed differences in LH and testosterone levels were due primarily to differences in food availability. It is more likely that the high population density and thereby the increased competition for breeding territories (including an increased frequency of aggressive encounters at the feeders) were the direct causes of the high LH and testosterone concentrations in the plasma of male Willow Tits from the fed population. This opinion is in agreement with the results of Wingfield (1984, 1985c) showing that stimuli emanating from other males in connection with territorial establishment may act as essential supplementary information.

One conclusion from our studies must be: LH secretion in Willow Tits *can* increase at the same time as in Great Tits and Crested Tits. Then why didn't we observe a rise in LH secretion in Willow Tits at 11 hr of light per day in the laboratory experiment? This might be due to a latency in the photoperiodic response of the Willow Tit or to a difference between the species in the rate to which they respond to the same day length ("the critical photoperiod") in spring. Furthermore, it seems as if certain environmental factors, e.g., competition for a resource, can affect the photoperiodic response.

Why did not the Crested Tit, which has the same autumn/winter ecology as the Willow Tit, show the same LH pattern as the Willow Tit in the laboratory experiment, and why did not the Great Tit, which breeds even somewhat later than the Willow Tit, show the same response in plasma levels of LH as did the Willow Tit? The answers to these questions are most likely connected with the evolution of the breeding seasons. As the birds followed the melting inland-ice towards the north there was a selection for egg laying as soon as possible after the activation of the reproductive system in Crested Tits. Although all tit species have basically the same LH secretion and gonadal growth patterns, there was, for some reason, selection for later onset of egg laying in Great Tits and Willow Tits. However, male Great Tits, and under certain circumstances also Willow Tits, still utilize their inherited ability to increase LH and testosterone secretion early in spring, not to start nesting, but in order to secure a good breeding territory and to attract a female to the territory as early as possible in the season. Once in possession of these resources, environmental factors act upon the female to determine the onset of the actual breeding cycle, and these factors must differ in some respect between the species.

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