

## Endocrine correlates of the breeding asynchrony between two corsican populations of blue tits (*Parus caeruleus*)

Samuel P. Caro<sup>a,b</sup>, Jacques Balthazart<sup>a,\*</sup>, Donald W. Thomas<sup>c</sup>, André Lacroix<sup>d</sup>,  
Olivier Chastel<sup>d</sup>, Marcel M. Lambrechts<sup>b</sup>

<sup>a</sup> Center for Cellular and Molecular Neurobiology, Research Group in Behavioural Neuroendocrinology, Univ. of Liège,  
17 Place Delcour (Bât. L1), B-4020 Liège, Belgium

<sup>b</sup> Centre d'Ecologie Fonctionnelle et Evolutive, Centre National de la Recherche Scientifique, 1919 Route de Mende,  
F-34293 Montpellier Cedex 5, France

<sup>c</sup> Groupe de Recherche en Ecologie, Nutrition et Energétique, Centre de Recherche en Biologie Forestière,  
Univ. de Sherbrooke, Sherbrooke, Que., Canada J1K 2R1

<sup>d</sup> Centre d'Etudes Biologiques de Chizé, Centre National de la Recherche Scientifique, 79360 Villiers en Bois, France

Received 22 April 2004; revised 9 September 2004; accepted 27 September 2004

### Abstract

Analyses of the development of the reproductive system in seasonally breeding birds in the framework of long-term ecological studies are rare. Here, we present the first results of such a study in two Corsican populations of a European passerine bird, the blue tit (*Parus caeruleus*). The two study populations occupy different oak habitats and are separated by only 25 km. Despite their close proximity, they show a one-month difference in onset of egg laying, even after controlling for altitude. This micro-geographic difference in breeding date appears adaptive because both study populations raise chicks when food is most plentiful. In our study, males reached their maximum song activity during the egg-laying stage while maximal testosterone levels and testes sizes were reached 2–3 weeks before egg laying. The rate of development of the reproductive system in males was much faster in the earlier population, in spite of a similar onset of gonad development and song activity for the two study populations. No change in the volume of the song-control nuclei (HVC and RA) could be detected during the study period. Development of brain nuclei was completed 2–3 months before the beginning of intense sexual activity.

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**Keywords:** Testosterone; Bird; Testes; Song nuclei; Season; Blue tit; *Parus caeruleus*; Mediterranean; Reproduction; Population

### 1. Introduction

Seasonally breeding birds are subject to strong selection pressures to match their reproduction with the optimal breeding time, which usually occurs during a short period of the year when food required for fast-growing offspring is most abundant. Pairs raising chicks well before or after the optimal breeding period may pay

considerable energetic or fitness costs and should therefore be counter-selected across generations (Lack, 1968; Murton and Westwood, 1977; Thomas et al., 2001; Van Noordwijk et al., 1995; Visser and Lambrechts, 1999; Wingfield and Moore, 1987).

The proximate challenge for seasonally breeding birds is therefore to defend resources, obtain a partner, grow gonads, and develop eggs in such a way that chicks will be in the nest when food is most abundant (Murton and Westwood, 1977; Wingfield et al., 1997). The preparation of the reproductive system functioning in a seasonal environment involves a series of rapid changes

\* Corresponding author. Fax: +32 4 366 59 71.

E-mail address: [JBalthazart@ulg.ac.be](mailto:JBalthazart@ulg.ac.be) (J. Balthazart).

at the physiological, morphological, and behavioral levels. In the north-temperate zone, many male birds show a vernal increase in singing behavior that is used to acquire and defend a territory and/or to attract potential mates (Catchpole and Slater, 1995; Kroodsma and Miller, 1996). These behavioral changes result from alterations in the activity of hypothalamo–pituitary–gonadal axis. For some species, the growth of specific brain nuclei apparently mediates the development, production and organization of bird song behavior (Ball et al., 2002; Brenowitz, 1999; Brenowitz and Kroodsma, 1996; Ritters et al., 2000; Smith et al., 1997a; Tramontin and Brenowitz, 2000). These morphological brain changes are generally under the influence of the gonadal secretion of testosterone (Smith et al., 1997a). In parallel, male aggression often peaks at the same time as testosterone levels, and song activity (Wingfield et al., 1994). All these events must thus be precisely timed in order for birds to be reproductively ready at the right time (Wingfield and Moore, 1987).

Multi-disciplinary investigations of the development of the reproductive system in the framework of long-term ecological studies that simultaneously quantify selection pressures and fitness consequences of the timing of reproduction are rare (Lambrechts and Visser, 1999). Here, we present the first results of such a study on population in Corsican blue tits (*Parus caeruleus*). These birds are exposed to a pronounced seasonal environment in which chicks can be raised in optimal conditions during a 2–3 week period only, when defoliating caterpillars, key prey for raising chicks, are most plentiful (Banbura et al., 1999; Blondel et al., 1993, 1999; Dias and Blondel, 1996a,b; Lambrechts et al., 1997a; Tremblay et al., 2003; Zandt et al., 1990). Chicks that are raised before or after the peak date of caterpillar availability show reduced survival and fledging mass (Tremblay et al., 2004) and parents pay a higher cost for nestling provisioning (Dias and Blondel, 1996a,b; Thomas et al., 2001).

In the Mediterranean region, blue tits are exposed to heterogeneous landscapes, differing in the availability of broad-leaved deciduous (*Quercus humilis*) and evergreen (*Quercus ilex*) oak trees. In broad-leaved deciduous oak, caterpillars develop one month earlier than in evergreen oak. Landscapes dominated by broad-leaved deciduous oak should therefore select for early breeding whereas landscapes dominated by evergreen oak habitat should select for late breeding (Blondel et al., 1993, 2001; Dias and Blondel, 1996b; Lambrechts and Dias, 1993; Lambrechts et al., 1997a,b). Long-term ecological studies in Corsica show that blue tit populations from different valleys, although they are situated at similar latitudes and altitudes, can show considerable differences in the timing of reproduction, even at micro-geographic scales (Blondel et al., 1999, 2001; Lambrechts et al., 1997a, 1999). In one valley (Muro), blue tits raise chicks in May at the time when many caterpillars are available in broad-leaved

deciduous oak habitat. By contrast, in another valley (Pirio), blue tits raise chicks in June at the time when most caterpillars are available in evergreen oak. Extensive ecological studies spanning more than a decade showed that blue tit populations from these two valleys differ not only in timing of reproduction, but also in other phenotypic traits (Blondel et al., 1999; Braillet et al., 2002; Doutréant and Lambrechts, 2001; Lambrechts et al., 1997a). Moreover, no exchange of marked reproducing birds has been observed between the two study sites during a 10-year study period. Between-valley exchanges of reproducing individuals are therefore assumed to be low, favouring local adaptation (Blondel et al., 1999).

The aim of the present study was to quantify the development of the gonads and song control nuclei, changes in testosterone levels and song activity in these two Corsican blue tit populations. We predicted an earlier development of the reproductive system in the blue tit population adapted to broad-leaved deciduous oak than in the blue tit population adapted to evergreen oak, despite a short geographical distance (25 km) between the two study populations.

## 2. Methods

### 2.1. Study sites

Blue tit populations from the broad-leaved deciduous (Muro) and evergreen (Pirio) sites were studied using basic protocols established since 1976 (see Blondel et al., 1999; Lambrechts et al., 1997a; for details). Both sites are located at similar latitudes (Muro: 42°32' north; Pirio: 42°22' north) and altitudes (Muro: 280 m; Pirio: 200 m; see: Lambrechts et al., 1997a, 2004). All data on the onset of egg laying, song activity, testosterone levels and testis volume were collected on both sites during the 2002 breeding season. Birds were killed following the ethical guidelines of the CNRS, with certificate No. 34–96 provided by the French Ministère de l'Agriculture et de la Forêt.

### 2.2. Song activity

Male song activity was measured from the end of February until the time when late females started laying eggs using the point counts method (described by: Blondel, 1975; Blondel et al., 1970, 1981). Sampling of singing activity started 30 min before sunrise and was conducted during four 10-min point counts. Each 10-min sampling period was divided into 10 one-minute periods, during which the presence/absence, position and vocal activity of males was noted. The total singing activity in a sampling day was evaluated by summing the scores of the different males heard during the four point counts. For the final analyses of song activity patterns, 1–4 sampling

dates were pooled, with data from the four point counts kept separate, to obtain comparable mean sampling dates in each study site (see Fig. 1 legend for additional details on data processing).

### 2.3. Blood sampling and testosterone assays

Males were trapped every two weeks until 5–11 individuals per study site had been captured. Birds were caught with mist nets after being attracted by playing tape-recorded blue tit songs and by the presence of live blue tit decoys around the net. All birds were caught before 13:00 h. Two to ten minutes following capture, a blood sample (200  $\mu$ l) was taken from the jugular vein in heparinized syringes (Omnican 30, B. Braun; Melsungen, Germany).

The time of exposure to the recorded song and to the decoy were always kept to a minimum and very few aggressive interactions could take place between the experimental subject and the decoy to minimize potential effects on plasma steroid levels. Based on previously published work on other songbirds (e.g., Wingfield, 1985), it is therefore very likely that, in these conditions, the capture technique did not interfere with the plasma levels of T that were measured. However, even if this was the case, the effect should be similar in both populations and would therefore not affect the *relative* patterns of seasonal changes. Samples were immediately centrifuged in the field and plasma was isolated. Samples were then frozen on dry ice ( $-80^{\circ}\text{C}$ ) and stored in freezers ( $-40$  or  $-80^{\circ}\text{C}$ ) until the hormone assays.

Testosterone titers were determined by radioimmunoassay (RIA). Protocols were developed and validated for birds at the CEBC (Centre d'Etudes Biologiques de Chizé) (as detailed in Chastel et al., 2003; Mazuc et al., 2003). Testosterone was extracted from a 50  $\mu$ l plasma sample in 3 ml diethyl-ether and assayed in duplicate in one single assay for all samples. Extracts were re-dissolved in 0.3 ml of 0.01 M phosphate-buffered saline (pH 7.4) containing 0.1% bovine serum albumin (PBS–BSA). Two aliquots of 100  $\mu$ l were incubated overnight at  $4^{\circ}\text{C}$  with 100  $\mu$ l (ca. 9000 cpm) of tritiated testosterone (TRK 921, 250  $\mu\text{Ci}$ , Amersham-France) and 100  $\mu$ l of specific antiserum (Dr. G. Picaper, CHR Orleans, France). The free and bound steroids were separated by addition 0.5 ml of dextran-coated charcoal. After centrifugation, which precipitated free steroids, radioactivity in the upper phase was measured in a liquid scintillation counter (1600 Packard). The lowest concentration detectable was 0.1 ng/ml and the intra-assay coefficient of variation was lower than 5%. The antiserum used for these assays does not cross-react significantly with any other steroid except for a cross-reaction of 30% with  $5\alpha$ -dihydrotestosterone (specification sheets of the company, confirmed by test trials in our laboratory). Levels of  $5\alpha$ -dihydrotestosterone are usu-

ally quite low compared to testosterone in songbirds (Wingfield and Farner, 1975, 1978) and they tend to covary, in a dampened mode, with changes in plasma T (e.g., Tanvez et al., 2004; Wingfield and Farner, 1978). The limited interference of  $5\alpha$ -dihydrotestosterone in the assays therefore should not affect the seasonal patterns of changes and in particular the difference in phenology between sites which is the focus of the present study.

### 2.4. Sampling and treatments of male brains

Male brains and testes were collected at the end of February and March in both study sites. Another sample was taken in the evergreen site at the end of April, before egg laying. These sampling periods were assumed to cover the time of rapid gonad development in the two study populations. Males feeding chicks were never taken to avoid chick loss. Trapping on the study sites used for long-term studies was avoided. The number of males was kept to a minimum compatible with the statistical analysis of the data (6–7 per date and site) to minimize animal suffering and to avoid depleting the local populations.

Males were caught using the same methods as those used for blood sampling. A blood sample (300–450  $\mu$ l) was taken as soon as possible after birds were trapped and males were killed by decapitation. Plasma samples were added to the samples collected for hormonal assays. Brain and testes were dissected out and immersed in a fixative solution made of phosphate-buffered saline (PBS) containing 5% Acrolein (Acros Organics; Geel, Belgium) for 4 h:1 h first without agitation and 3 h under agitation. Before fixation, testes were measured (length and width) with a precision of 0.1 mm and their volume calculated using the equation of the spheroid:  $V = 4/3\pi\alpha^2\beta$ , where  $\alpha$  is half the testis width and  $\beta$  is half the testis length. Tissues were cryoprotected in a 30% sucrose solution overnight and frozen on dry ice ( $-80^{\circ}\text{C}$ ) until analyses.

Brains were cut in 30  $\mu\text{m}$  transverse frozen sections on a microtome. Every fifth section was mounted on gelatin-coated slides, and the others were stored at  $-20^{\circ}\text{C}$  in cryoprotectant solution. Mounted sections were Nissl-stained (Nissl, 1894) with toluidine-blue to allow the identification and measure of various cerebral structures, including song control nuclei. The volumes of HVC (used as a name, formerly the “High Vocal Center”) and RA (Robust nucleus of the Arcopallium, formerly Archistriatum; see <http://avianbrain.org> for current discussions on the update of the avian neuroanatomical nomenclature) were measured for the present study. Images of the histological sections were captured by a video camera connected to a microscope using the computer software NIH Image version 1.52 (Wayne Rasband, NIH, Bethesda MD, USA). Nissl-defined borders

of the nuclei were delineated with the computer mouse on the screen and surfaces were measured by the program. The limits of HVC are easily identified by the presence of large, darkly stained cells relative to the surrounding nidopallium (Bernard and Ball, 1995). The limits of RA are sometimes more difficult to delimit; they were identified partly thanks to larger cells in periphery and to a lamina which partially surrounds the nucleus on its rostro-caudal extension. Area *X* was not measured because its boundaries are too difficult to resolve in blue tits (Absil P., pers. comm.). The nuclei volumes were then reconstructed by multiplying the surface of each section by the sampling interval. No left–right difference in HVC and RA volumes was found, so all nuclei volumes are reported here as a mean of both sides. HVC and RA volumes are expressed here in cubic millimeters and not corrected for the entire brain volume since numerous studies have previously demonstrated the absence of significant changes in the total volume or weight of the brain, of the telencephalon or of parts of the telencephalon in songbirds (e.g., Ball et al., 2004; Brenowitz et al., 1998; Smith et al., 1997b; Tramontin et al., 1999, 2001). Changes in the volume of song control nuclei are specific and do not affect other non steroid-sensitive areas (e.g., Ball et al., 2004; Brenowitz et al., 1998; Tramontin et al., 1999).

### 2.5. Statistical analyses

Differences between study populations (Pirio vs. Muro) and effects of the sampling time on the different components of the reproductive system (song, testis size, brain nuclei size, and testosterone levels) were tested using two-way ANOVAs. Post hoc comparisons were carried out with Fisher protected least significant difference (PLSD) tests to compare the samples two by two. Egg laying dates were compared using a Mann–Whitney *U* test. Effects were considered significant for  $P \leq 0.05$ .

## 3. Results

### 3.1. Onset of egg laying

In agreement with previous studies on these populations (Blondel et al., 1999, 2001; Lambrechts et al., 1997a, 1999), egg laying in 2002 started and ended earlier in the Muro than in the Pirio population. Between-population differences in the average onset of first clutches were highly significant ( $U=0$ ,  $P<0.0001$ ) (Fig. 1). The latest female from Muro finished egg laying of first clutches before the earliest female started egg laying in Pirio, so that there was no overlap in egg laying dates between the two populations.

### 3.2. Song activity

Singing activity varied seasonally in both study sites but peak levels were not observed in synchrony (Fig. 1). ANOVA of these data indicated that song activity was significantly influenced by the interaction between study sites and time of the season ( $F_{9,60} = 3.268$ ,  $P = 0.003$ ). The overall effect of the study site on song activity was also significant ( $F_{1,60} = 4.509$ ,  $P = 0.038$ ) but not the effect of time ( $F_{9,60} = 1.742$ ,  $P = 0.099$ ). The seasonal peak in singing activity occurred 26 days later in the evergreen site than in the broad-leaved deciduous site (May 9 vs. April 13), which corresponds very closely to the time when females started egg laying in each study population (see arrows in Fig. 1).

### 3.3. Testosterone levels

Plasma levels of testosterone varied significantly during the breeding season in male tits of both populations ( $F_{4,64} = 2.645$ ,  $P = 0.042$ ). Overall testosterone levels were not significantly affected by the study site ( $F_{1,64} = 0.014$ ,  $P = 0.905$ ), but the interaction between study sites and sampling dates was significant ( $F_{4,64} = 4.170$ ,  $P < 0.005$ ). Seasonal plasma testosterone profiles were similar in both populations but simply delayed in time in Pirio (Fig. 2). In both populations, the dates with maximum testosterone levels occurred during the nest building stages (Muro: March 25 vs. Pirio: April 20).

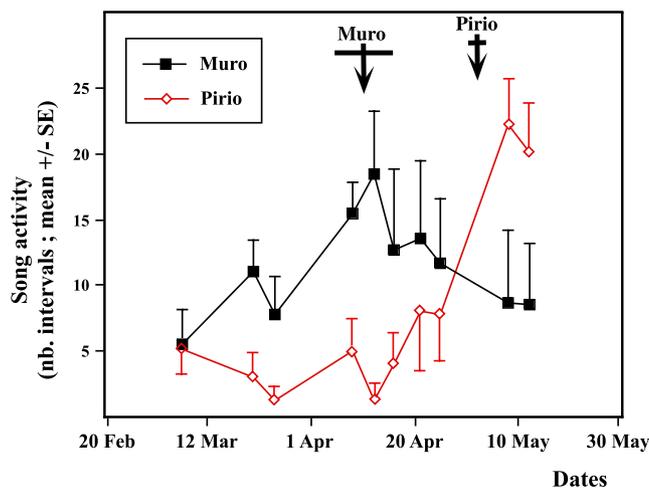


Fig. 1. Seasonal changes in total song activity in the two Corsican blue tit (*Parus caeruleus*) populations. The graph presents the total number of song intervals heard during four point counts in the two study sites (Muro and Pirio) from the beginning of the breeding season until the egg laying period in the latest valley of Pirio. Data from several days were pooled into mean single point counts to construct curves including the same dates in both sites (10 pooled samples from 28 single days of recording). Arrows at the top of the curves show the median and interquartile range (horizontal bar) of the middle of the egg laying period. There is a close relation between female egg laying and male maximum song activity in each study site.

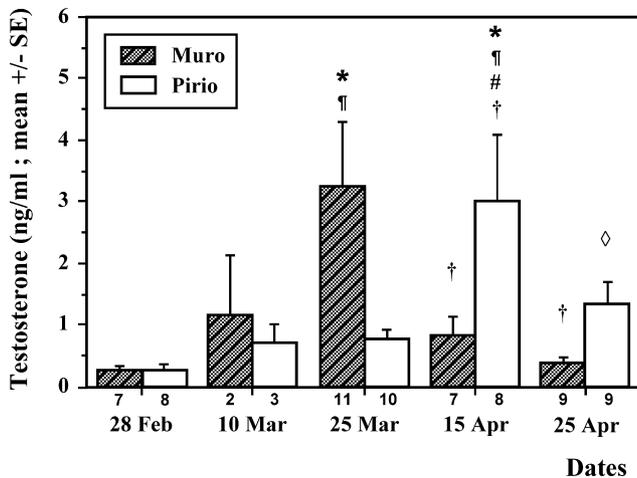


Fig. 2. Seasonal changes in plasma levels of testosterone in male Corsican blue tits at the two study sites of Muro ( $n = 36$  samples assayed) and Pirió ( $n = 38$  samples assayed). Data are presented as means  $\pm$  SE. The number of independent data points at each date and site is indicated below the corresponding bar. Results of post hoc comparisons following significant ANOVAs are represented at the top of the bars as follows: \* $P < 0.05$  by comparison with the other study site at the same date; † $P < 0.05$  by comparison with the same site on February 28; ‡ $P < 0.05$  by comparison with the same site on March 10; †† $P < 0.05$  by comparison with the same site on March 25; and ††† $P < 0.05$  by comparison with the same site on April 15.

### 3.4. Testis volumes

Testis volumes showed a marked seasonal growth in both populations (Fig. 3). Testis sizes were significantly affected by the sampling time ( $F_{2,26} = 48.552$ ,  $P < 0.001$ ), but not by study site ( $F_{1,26} = 2.008$ ,  $P = 0.17$ ) and the interaction between these two factors was not statistically significant ( $F_{1,26} = 2.020$ ,  $P = 0.17$ ). Both populations apparently started their rapid gonad development at a similar date after the second half of February. However, in both study populations, a slow growth of the testes already took place at the same rate between mid-December and February. Recent work indeed indicates that testes size is significantly smaller in Corsican blue tits in mid-December (combined data for both sites:  $0.56 \pm 0.135 \text{ mm}^3$ ;  $n = 9$ ; means  $\pm$  SD) than in the samples collected in February for the present study (combined data for both sites:  $4.03 \pm 2.58$ ; means  $\pm$  SD;  $n = 15$ ;  $F_{1,20} = 65.054$ ,  $P = 0.0013$ ) but there is no difference between sites and no interaction between these two factors ( $P > 0.92$  in the two way ANOVA; S.P. Caro, J. Balthazart, and M.M. Lambrechts unpublished data). However, the speed of testis development increased very markedly after the end of February and was much higher (almost double) in the early deciduous than in the late evergreen site. Finally, males sampled just before egg laying in the deciduous site (28 March) had significantly smaller gonads than males sampled just before egg laying in the evergreen site (28 April) ( $P < 0.001$ ).

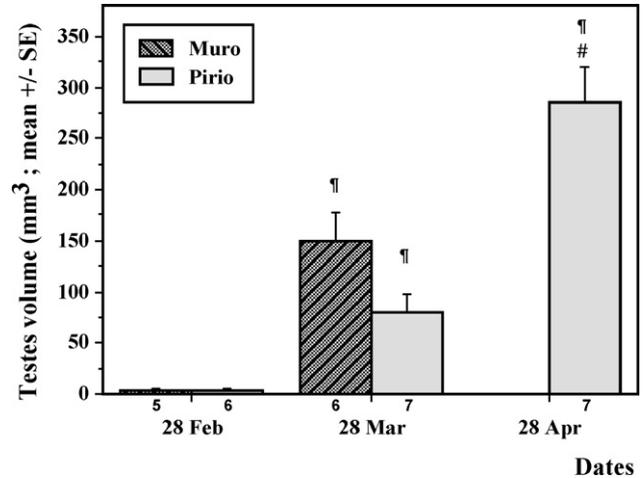


Fig. 3. Seasonal changes in the testes volumes of Corsican male blue tits at the study sites of Muro and Pirió. Data represent the total testes volumes obtained by addition of the volumes of the left and right testes and are presented as means  $\pm$  SE. The number of independent data points at each date and site is indicated below the corresponding bar. Results of post hoc comparisons following significant ANOVAs are presented by symbols at the top of the bars as follows: † $P < 0.05$  by comparison with the same study site on February 28; †† $P < 0.05$  by comparison with the same site on March 28; ††† $P < 0.05$  by comparison with the same site on April 28.

### 3.5. Song control system morphology

No significant effect of the study site and of the time of the season on the size of HVC and RA could be detected (Study site: HVC  $F_{1,23} = 2.486$ ,  $P = 0.129$ ; RA  $F_{1,23} = 0.076$ ,  $P = 0.786$ –Time: HVC  $F_{2,23} = 1.281$ ,  $P = 0.30$ ; RA  $F_{2,23} = 0.381$ ,  $P = 0.69$ ). There was also no interaction between study sites and time of the season (HVC  $F_{1,23} = 0.506$ ,  $P = 0.48$ ; RA  $F_{1,23} = 2.019$ ,  $P = 0.17$ ). Thus, in both study populations, the size of the brain song control nuclei did not vary during the two-month sampling period (Fig. 4).

## 4. Discussion

Previous work on the two Corsican blue tit populations studied here showed remarkable differences in many phenotypic traits measured in the late phases of the reproductive cycle, including major life-history traits such as the timing and effort of reproduction, breeding success, song dialects, and morphometry (Blondel et al., 1999, 2001; Braillet et al., 2002; Doutrelant and Lambrechts, 2001; Lambrechts et al., 1997a, 1999). Here, we show that these two populations also differ in the timing of the development of some aspects of the reproductive system prior to egg laying, as measured by singing activity, testosterone levels, and gonad development.

Testosterone has a major impact on different morphological and physiological features related to the

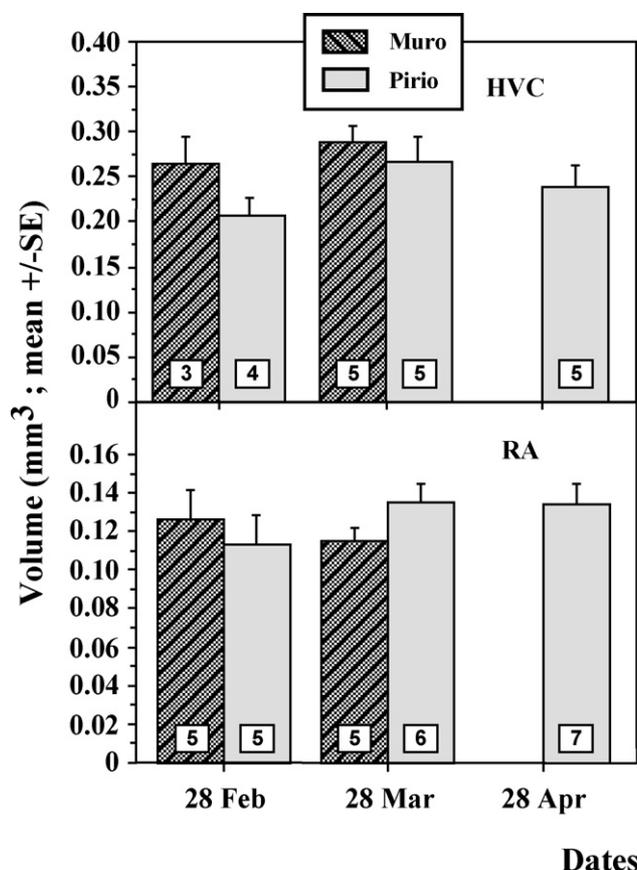


Fig. 4. Seasonal changes in the volume of two song control nuclei, HVC and RA in Corsican male blue tits at the study sites of Muro and Pirio. Data are presented as means  $\pm$  SE. The number of independent data points at each date and site is indicated in the corresponding bar. Two-way ANOVA revealed no significant difference related to the study sites, sampling dates or their interaction.

preparation of reproductive activities (Ketterson and Nolan, 1992). Testosterone (1) promotes the growth of the telencephalic song control nuclei (Bernard and Ball, 1995; Smith et al., 1997b), (2) induces the development of the syrinx muscles involved in song production (Lieberburg and Nottebohm, 1979; Tramontin et al., 2001), (3) stimulates singing activity and song structure development (De Ridder, 2002; Foerster et al., 2002; Schlinger and Brenowitz, 2002), and (4) increases male aggressiveness in response to potential rivals (Balthazart, 1983; Ketterson and Nolan, 1992; Wingfield et al., 1997). In the temperate zone, seasonally breeding males therefore often present one or two peaks of plasma testosterone levels, related either to territorial behavior in the beginning of the breeding season and/or to mate guarding at the time of female fertility (Wingfield, 1984). However, in the two blue tit populations studied in Muro and Pirio, we observed only a single testosterone peak approximately two/three weeks before the middle of egg laying and well before the time when males sang the most. This asynchrony suggests that intense singing activity and mate guarding behavior

during the egg laying period can be expressed in the absence of very high androgen levels. High song activity with basal testosterone levels has similarly been reported in starlings, *Sturnus vulgaris* (Pinxten et al., 2002). A “pre-laying” peak of testosterone as described in the present study was also observed in another blue tit population (Foerster et al., 2002), in great tits, *Parus major* (Van Duyse et al., 2003), feral pigeons, *Columba livia* (Murton and Westwood, 1977), starlings (Dawson, 1983; but see De Ridder, 2002) and mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha* (Morton and Allan, 1990; Morton et al., 1990). In all these studies including the present one, testosterone peaked during the nest-building period, suggesting that this period could represent a crucial time for the defense of important resources such as nest sites. Because females are involved in nest site choice, sudden shifts in territory boundaries could occur around the nest building phase, possibly stimulating territorial encounters between neighbors which secondarily would lead to the increases in plasma testosterone levels detected here.

Male blue tits in the two populations displayed pronounced differences in the speed of testicular development and in maximum testis size during the egg laying period. Although our two study populations started testis growth at a similar date (between December and February), males from the early deciduous habitat grew their gonads at a faster rate than males from the late evergreen habitat between the end of February and April. Testis growth (between the low values observed in late February until the egg laying stage) lasted one month longer in the evergreen than in the deciduous forest population. In addition, absolute testis size around the time of egg laying was larger for the evergreen than for the deciduous blue tit population. This latter difference is likely explained by the fact that more first-year birds were sampled in the deciduous (9 out of 11) than in the evergreen (4 out of 20) site at the time of egg-laying, and it is known in several species that first-year birds reach smaller final testis sizes than adult birds (Absil et al., 2003; Morton and Allan, 1990; Morton et al., 1990).

Analyses of the song control nuclei volumes failed to show any significant variation in size during the three months sampling period covering most of the spring. Two hypotheses could explain this finding. First, Corsican blue tits may not display any seasonal variation in their song control nuclei, which would maintain the same size all year round despite the presence of major changes in testes size, and plasma testosterone levels. Tits maintain territories during a large part of the annual cycle, with individuals sometimes singing in winter when photoperiod is short and ambient temperatures are low. However, based on former studies, this hypothesis appears unlikely (Schlinger and Brenowitz, 2002). In

addition, both in Belgian blue tits and Swedish great tits a vernal increase in the HVC and RA volumes has been observed (Absil et al., 2001; Silver et al., 2003). A second explanation for the lack of changes in the present study therefore is that the seasonal growth of the song nuclei occurred before the sampling period. The HVC and RA volumes measured here indeed correspond to fully developed volumes in the study of Belgian blue tits (Absil et al., 2001). Early growth of the brain nuclei controlling song has already been reported in song sparrows, *Melospiza melodia* (Smith et al., 1997a; Tramontin et al., 2001) and great tits (Silver et al., 2003). In song sparrows, HVC and RA were completely developed at the end of February before the beginning of the reproductive season. In the great tit study, song nuclei growth was observed as soon as January. The song control nuclei development thus took place in these species in the absence of high androgen levels in the blood (Tramontin et al., 2001). Although testosterone is often regarded as the primary physiological factor promoting the development of these nuclei (Absil et al., 2003; Brenowitz and Kroodsma, 1996; Smith et al., 1997a,b), other factors such as photoperiod or social factors are likely to induce their growth, even in the absence of steroids (castrated birds) (Ball et al., 2002; Bentley et al., 1999; Bernard et al., 1997; Smith et al., 1997b; Tramontin et al., 1999). Additional sampling during other periods of the year will be required to discriminate between these two hypotheses in our study system and to investigate whether or not the two blue tit populations differ in the onset or speed of brain nuclei growth patterns early in the season. Ongoing studies already indicate that smaller volumes of HVC and RA can be observed in Corsican blue tits in December thus clearly suggesting that song control nuclei show seasonal variations in volume in Corsica also but that the vernal growth occurs very early during the season.

The factors controlling the differences in breeding phenology in these two blue tit populations living at similar latitudes and altitudes still remain largely unknown but the present data together with previously published studies help narrow the range of possibilities. Key factors that are known to determine the timing of reproduction in seasonal environments include the photoperiod, food availability, temperature, and various social factors, such as interactions between males and sexual influences of partners. Several of these factors help to predict the optimal breeding time (e.g., Murton and Westwood, 1977; Wingfield and Moore, 1987; Wingfield and Silverin, 2002; Wingfield et al., 1997, 1998). A series of aviary experiments in combination with a long-term field program showed that large differences in the onset of egg laying between mainland blue tit populations adapted to early broad-leaved deciduous oak habitat and Corsican blue tits adapted to late evergreen oak habitat could be attributed to genetic differences in photoresponsiveness

(Lambrechts et al., 1996, 1997b; Lambrechts and Dias, 1993; Lambrechts and Perret, 2000). Population differences in photoresponsiveness would have evolved to anticipate highly predictable differences in optimal breeding time between the broad-leaved deciduous and evergreen oak habitats. Based on these former blue tit studies, we expected large genetic differences in male photoresponsiveness causing population differences in onset of development of song control nuclei in the brain and the onset of testes growth. Our results did not support this hypothesis because the onset of testis development started at similar dates for both blue tit populations. Moreover, brain song control nuclei involved in the control of song activity in males probably developed very early in the season well before we started sampling subjects in the present study, even in the population breeding late in evergreen habitat. This suggests that male territorial behavior may be important early in the season in both blue tit populations, independent of the timing of the optimal breeding date. Because males in both populations apparently initiated their gonadal development at the same early time, under the same photoperiod, these data also suggest that the asynchrony between the observed patterns of singing activity and testis development is probably due to population differences in male responses to “supplementary non-photoperiodic factors,” such as population differences in food availability required for gonad and/or egg formation and/or social stimuli (Wingfield and Moore, 1987).

Corsican blue tit pairs originating from the evergreen valley in Corsica (Pirio) breed at the same dates in captivity as in the wild (Lambrechts and Dias, 1993; Lambrechts and Perret, 2000) even when held with food available ad libitum, at similar latitudes and altitudes as their wild counterparts (see also Braillet et al., 2002). A direct effect of higher food constraints in the evergreen habitat therefore probably cannot explain the retarded testis development and breeding in the “evergreen” blue tit population. Social factors can also play a major role in the stimulation of rapid testis development, in particular sexual signals provided by the female to the male (Cheng, 1979; Hinde, 1965). Thus, it appears likely that differences in the speed of testis development observed between the two male populations could be a consequence of genetically based population differences in the timing of sexual development and egg laying in females. Sexual stimuli accelerating testis development in blue tit males would occur earlier in the “deciduous” than in the “evergreen” population, perhaps as a consequence of population differences in female photoresponsiveness. Additional experiments with captive populations kept in controlled conditions in aviaries will now be required to test the specific roles of males and females in the determination of population differences in timing of reproduction in this model system.

## Acknowledgments

We thank Nathalie Pelletier, Francis Gallant and Valérie Roy for their precious contribution to fieldwork. This study was supported by Grants from the NINDS (NS 35467), the Belgian FRFC (2.4555.01), the French Community of Belgium (ARC 99/04-241) and the University of Liège (Crédits spéciaux) to J.B., grants from the European Commission (METABIRD) and the CNRS to M.M.L., a travel grant from the FNRS to S.P.C., and grants from the Natural Sciences and Engineering Research Council of Canada to D.W.T., S.P.C. is a FRIA grant recipient.

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