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Local adaptation of timing of reproduction: females are in the driver's seat

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Summary

1. Breeding at the right time of the year is crucial for many temperate zone organisms, as any mismatch with their optimal breeding period leads to a reduction in fitness. The roles of the breeding partners in these adaptive decisions have, however, only rarely been studied.

2. In blue tits (*Cyanistes caeruleus*) that breed in heterogenous Mediterranean habitats, nearby populations show up to 1-month differences in breeding time. This difference is adaptive as the optimal time for breeding varies by the same amount due to habitat characteristics. But which sex is determining this large difference in timing?

3. We show here that the seasonal development of female reproductive activity (yolk precursor production) closely matches the local optimal breeding time in these two highly contrasted landscapes, while previous studies have demonstrated that male gonadal development does not. In accordance with this, quantitative genetic analyses reveal that timing for breeding is determined by the female only, with no evidence for an additive genetic male effect on laying date.

4. Our results strongly suggest that the adaptive inter-population difference in the laying periods has been driven by females. The central role of females in the determination of breeding period needs to be included when studying how micro-evolutionary processes are affected by global climate change.

Key-words: gonadal development, heritability, local adaptation, *Parus*, *Parus caeruleus*, timing of breeding, yolk precursors

Introduction

Successful breeding requires that birds closely match their breeding period with the brief moment when food is most abundant (Lack 1968; Murton & Westwood 1977; Balthazart 1983). Mistiming between reproduction and maximal food availability leads to fitness reduction (Thomas *et al*. 2001; Visser *et al.* 2006). As a consequence, the timing of reproduction is often considered as one of the major life history traits reflecting the adaptation of birds to local characteristics of their environment (Lambrechts & Visser 1999). Few long-term field studies have simultaneously investigated both proximate and ultimate factors governing this adaptation (e.g. Nager & van Noordwijk 1995; Ketterson *et al*. 2000), even if there is growing interest for the comparative study of local free-living populations (Silverin 1998; Tramontin *et al*. 2001; Leitner *et al.* 2003; Perfito *et al*. 2004; Moore *et al.* 2005; Partecke *et al.* 2005). In particular, we do not know whether selection pressures acting on the time of breeding are the same for both sexes. Selection pressures for matching the chick rearing period with the annual caterpillar peak should be acting on both feeding parents. However, because egg laying and incubation periods are largely constrained by high energetic and fitness costs (Perrins 1970; Monaghan & Nager 1997; Monaghan *et al.* 1998; Bryan & Bryant 1999; Visser & Lessells 2001; Vezina & Williams 2005), and because females of many bird species carry most of these costs alone, we predict that selection pressure should act more powerfully in females than in males (see e.g. Ball & Ketterson 2008).

Female blue tits (*Cyanistes caeruleus*, formerly *Parus caeruleus*) produce and incubate alone the largest relative clutch size of

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any nidicolous bird in the world (Perrins & McCleery 1989). In a species weighing 10 g at most, the mass of a single egg represents around 15% of the total body mass, and clutch size ranges from 6 to 9 eggs in Corsica and up to 12 eggs in central Europe' so that the entire clutch mass is in the same range as the adult bird weight. In addition, unusually high energy consumption is necessary during incubation for heating such large clutches (Walsberg 1983; Bryan & Bryant 1999; Cichon 2000). This makes the costs of egg production and incubation particularly high in tits, and therefore adds further importance to the determination of appropriate breeding time (Visser & Lessells 2001).

In the Mediterranean region, several blue tit populations that breed at similar latitudes and altitudes, and are therefore exposed to the same photoperiods, differ in timing of breeding by 1 month (Blondel *et al.* 1999). Numerous studies have demonstrated that this differential timing is adaptive and occurs in response to a similar differential timing of local maximal caterpillar biomass (e.g. Lambrechts *et al.* 1997a; Blondel *et al.* 1999; Thomas *et al.* 2001). Experiments on pairs of captive blue tits have shown that this difference in timing of breeding persists under controlled conditions (Lambrechts & Dias 1993). Furthermore, when blue tit parents that normally lay in May in the field, lay eggs in late March in captivity due to exposure to long photoperiod, their chicks still lay in May the following year, when kept in captivity under natural photoperiod (M.M. Lambrechts, unpublished data). Altogether, these data demonstrate that the laying dates of these highly differentiated populations have a genetic basis. Other experiments manipulating photoperiod in captivity have suggested that the breeding differentiation between populations is proximately caused by variations in the response to photoperiod. Blue tit pairs breeding in June in late evergreen oak forests (e.g. 'Pirio' Corsican study site) would have a higher response threshold to daylength compared to blue tit pairs breeding in May in early deciduous oak forests (e.g. 'Muro' Corsican study site) (Lambrechts *et al.* 1997b; Lambrechts & Perret 2000; Caro *et al.* 2007). However, recent field studies of male Corsican blue tits demonstrated that the early stages of the seasonal recrudescence of the hypothalamo–pituitary–gonadal (HPG) axis occur simultaneously in males living in deciduous and evergreen oak forests (Caro *et al.* 2005a,b, 2006). Subsequently, the onset of rapid testis growth occurred with a maximum difference of only 1–2 weeks between the two populations (Caro *et al.* 2006). Similar observations were made for plasma testosterone. This suggests that the difference in photoperiodic response between males from the two habitats, if any, is very limited.

The present study aimed to describe and compare the timing of onset of rapid gonadal development in female Corsican blue tits from deciduous and evergreen habitats, and to assess the relative influence of each sex on laying dates in these locally adapted breeding populations. As social factors usually play an important role in synchronizing male and female reproductive development within pairs (Moore 1982; Jacobs & Wingfield 2000; Moore *et al.* 2005), the early sexual development previously observed in males could trigger a similar early recrudescence of female reproductive organs in the evergreen habitat and, as a consequence, a relatively synchronous follicular development between the habitats. In this case, the pre-breeding sexual development would also be relatively synchronous between males and females within each of these populations, a phenomenon that has often been predicted and/or observed in other species (e.g. Moore *et al.* 2005; Partecke *et al.* 2005). Alternatively, if females rely on other environmental cues that accurately reflect habitat phenologies, we should observe a complete differentiation of the rapid follicular developments between the two populations, and therefore an asynchronous rapid gonadal development between males and females in the evergreen habitat. To discriminate between these two scenarios, and decipher the respective roles of each sex in the determinism of this breeding asynchrony, we used two complementary approaches. First, we assessed and compared the seasonal reproductive development of females in the blue tit populations of deciduous (Muro) and evergreen (Pirio) habitats by measuring variation in yolk precursor levels (e.g. Challenger *et al.* 2001). As yolk precursors are oestrogen-dependent liver products (Walzem *et al.* 1999), a relative synchrony in female steroid recrudescence between Muro and Pirio, as observed in male plasma testosterone and predicted by our first hypothesis, would also induce a relative synchrony in the yolk precursor recrudescence, even if laying does not occur directly. Second, we performed linear mixed models and pedigree-based quantitative genetic analyses in these two populations to estimate sex-specific contributions to the variation in the timing of reproduction, including those mediated by additive genetic effects.

Methods

FIELD PROCEDURES

The study was carried out in two Corsican blue tit populations in broad-leaved deciduous (Muro) and evergreen (Pirio) habitats. All protocols used in the demographic study of these populations are described in Blondel *et al.* (1993). All breeding attempts in nest-boxes are monitored from nest building to fledgling, and all breeding individuals and nestlings uniquely identified. Plasma samples were collected from females during spring in 2003 and 2005 near the study plots of the long-term population studies as described in Caro *et al.* (2005a). A total of 173 blood samples, from 173 different females, were collected (116 in 2003 and 57 in 2005) over a 3 months period, before and during the breeding season (Fig. 1).

YOLK PRECURSOR ASSAYS

The seasonal growth and regression of the ovary can be reliably assessed by the measurement of the egg-yolk precursors, vitellogenin (VTG) and triglyceride-rich yolk targeted very-low-density lipoprotein (VLDL) produced by the liver in response to oestrogenic stimulation (Mitchell & Carlisle 1991; Vanderkist *et al.* 2000). VTG and VLDL levels are highly correlated and, in passerines, the seasonal increase, maintenance of elevated levels, and decrease in plasma levels of these yolk precursors are often tightly coupled to the timing of follicle

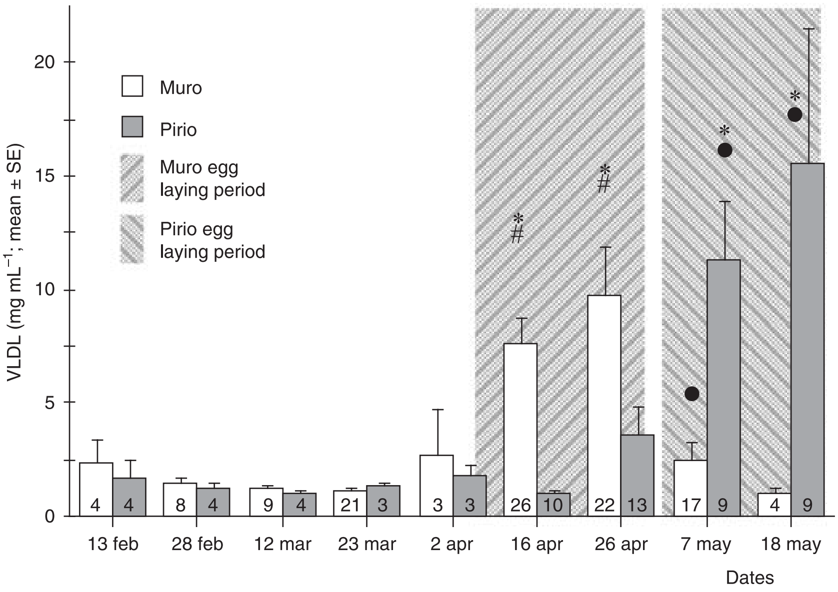

Fig. 1. Seasonal changes in plasma levels of very low density lipoproteins (VLDL) in female Corsican blue tits ($n = 173$ samples assayed) in Muro and Pirio. Data are presented as mean ± SE. Main results of post hoc comparisons following significant ANOVAS are represented at the top of the bars as follows: *$P < 0{\cdot}01$ by comparison with the other study site at the same date; **#**$P < 0{\cdot}001$ by comparison with the same study site on 23 March; •$P < 0{\cdot}01$ by comparison with the same study site on 26 April.

development (Challenger *et al.* 2001; Vezina & Williams 2003). However, several studies have shown that follicles start growing well before the egg laying period (e.g. Bissonnette & Zujko 1936) and that significant increase of yolk precursors in plasma can be measured before the onset of rapid follicular growth (Sockman *et al.* 2004), demonstrating that the yolk precursor recrudescence does not necessarily match the egg laying period. Because only small plasma volumes can be collected in blue tits and the VLDL assay is more sensitive than the VTG assay, we used VLDL as our measure of yolk precursor levels in this study. Plasma VLDL was measured as triglyceride levels (Mitchell & Carlisle 1991) using an analytical assay for free glycerol and total glycerol (Sigma-Aldrich, Canada) with plasma triglyceride calculated as the difference between total glycerol and free glycerol. Samples were assayed in triplicate and the inter-assay coefficient of variation, CV(%) was < 10% for free and total glycerol in both 2003 and 2005 (determined using a same plasma pool from a 19 week hen that was included in the 9 separate runs of each assay). Intra-assay CV% was 5·7% for free glycerol and 6·2% for total glycerol.

Differences between the two study populations (Pirio vs. Muro) and effects of the sampling date on plasma VLDL concentrations were analyzed by two-way ANOVAS. Partial analyses made on data from periods when samples had been collected in both years, failed to identify any difference between the 2 years ($P > 0{\cdot}05$, details not shown). Hence, data from the 2 years of study (2003 and 2005) were pooled. Post hoc comparisons were carried out with Fisher protected least significant difference tests. Egg laying dates were compared using a Mann–Whitney U-test. Effects were considered significant for $P \leq 0{\cdot}05$.

QUANTITATIVE GENETIC ANALYSIS

We used the pedigrees of social relationships from the long-term monitoring of Muro (1994–2007) and Pirio (1979–2007), along with the phenotypic records on egg laying dates during the same period in these two study sites, to decompose the breeding date of a pair into male and female phenotypic and genetic components. A quantitative genetic framework has been used before in these populations to estimate heritabilities of morphological traits (Charmantier *et al.* 2004a; Charmantier *et al.* 2004b). The pedigrees related 720 breeding birds over 7 generations in Muro and 1261 breeders over 12 generations in Pirio.

The purpose of this analysis was to decompose the roles of females and males in determining laying dates, and if possible to estimate the genetic covariance between laying dates in the two sexes. The decomposition of the variance in laying dates attributable to female and male effects was investigated using linear mixed effect models including female and male identity. By investigating male and female effects simultaneously, this single model partially accounts for not missing at random biases (Hadfield 2008) which can result, for example, from lower recapture rates in males compared to females (see Table 1). All models included year of breeding, age of the female and age of the male as fixed effects, and were solved using restricted maximum likelihood implemented in ASReml 2·0 (Gilmour *et al.* 2002). For birds first ringed as adults, the age was set to a minimum of 2 years at first capture. Additive genetic female or male components were then included, using the pedigrees of breeding blue tits in each studied population to fit a quantitative genetic 'animal model' (Lynch & Walsh 1998; Kruuk 2004). In all models, the significance of a variance component was estimated with a likelihood-ratio test (LRT) comparing the deviance of models including/excluding this component with a χ^2-distribution (d.f. = 1). Alternative models were selected using the standard Akaike's information criterion (AIC, Burnham & Anderson 2002).

Repeated records on breeding individuals allowed us to account for and estimate the variance due to persistent non-heritable differences between females and between males, hereafter called 'permanent female/male environment' effects (see Kruuk 2004), as well as variance due to additive genetic female or male effects. Hence the most complex mixed model tested in one population partitioned the total phenotypic variance (V_P) into:

$$V_P = V_{PEf} + V_{PEm} + V_{Af} + V_{Am} + V_R$$

where V_{PEf} and V_{PEm} are the variances due to permanent environment differences between females and males, V_{Af} and V_{Am} are variances due to additive genetic female and male effects, and V_R is the residual

Table 1. Sample sizes, mean laying dates, and divorce rates in two Mediterranean blue tit populations

Study site	Years of sampling	Number of records	Proportion with unknown male identity (%)	Proportion with unknown female identity (%)	Mean laying dates ± SD (1 = 1 March)	Number of breeding attempts where male and female survived to the next year	Divorce rate (%)
Muro	1994–2007	454	9·7	2·4	36·7 ± 8·4	276	38·3
Pirio	1979–2007	1228	8·7	4·7	70·5 ± 7·1	47	42·4

variance. One important advantage of a single model fitting both male and female effects it that is avoids the common drawback of V_{PEm} inflated by V_{PEf} and vice versa because of incomplete random mating, when fitting separate models.

Since we did not find any significant additive genetic male variance in laying dates, we could not test for a genetic covariance between laying date in the two sexes.

DIVORCE RATES

In the above model, permanent environment male and female effects can only be appropriately decomposed if individuals do not always mate with the same partners. Indeed, if divorce does not exist in our populations, then sex specific permanent effects V_{PEm} and V_{PEf} cannot be separated. We have estimated the rate of divorce in Muro and Pirio based on the demographic data collected up to 2007, considering only first brood attempts. When both birds in a pair were alive the year following their pairing, then they were divorced if one of them at least bred with another partner in that following year.

Results

VARIATION IN PLASMA YOLK PRECURSOR LEVELS

VLDL concentrations (Fig. 1) varied significantly during the breeding season ($F_{8,155} = 2{\cdot}636$, $P = 0{\cdot}0098$). The interaction between study sites and sampling periods was highly significant ($F_{8,155} = 5{\cdot}112$, $P < 0{\cdot}0001$) but there was no overall difference between sites ($F_{1,155} = 0{\cdot}645$, $P = 0{\cdot}4230$). Post hoc tests indicated that plasma VLDL concentrations were in general significantly higher in both locations during the egg laying period compared to the pre-laying period (from 16 to 26 April in Muro and from 7 to 18 May in Pirio; see Fig. 1). Post hoc comparisons between study sites showed differences exclusively during the laying periods. Together, these results demonstrated that both onset of VLDL increase and periods of VLDL maximal concentration were different and did not overlap between the two populations. The maximal levels of VLDL, observed during the respective egg laying periods (Muro: from 16 to 26 April; Pirio: from 7 to 18 May), did not differ but tended to be higher in Pirio than in Muro (one-way ANOVA: $F_{1,64} = 3{\cdot}326$, $P = 0{\cdot}0729$).

EGG LAYING PERIODS IN 2003 AND 2005

During the two study years, there was almost no overlap in the laying periods in Muro and Pirio: most females in Muro finished egg laying before the earliest female started laying eggs in Pirio (2003: $U = 0$, $n_{\text{Muro}} = 59$, $n_{\text{Pirio}} = 50$, $P < 0{\cdot}0001$; 2005: $U = 0$, $n_{\text{Muro}} = 44$, $n_{\text{Pirio}} = 56$, $P < 0{\cdot}0001$ for the median of the egg laying dates of each female). This non-overlap is also true when looking at the long-term monitoring in both populations (see Table 1).

DIVORCE RATES

Divorce rates were estimated at 42·4% in Pirio ($n = 117$ divorces out of 276 pairs where both birds were alive following their pairing, Table 1) and 38·3% in Muro ($n = 18$ out of 47). Hence, divorce rates were quite high in both populations and tend to be similar or higher than in other European populations of tits (Blondel *et al.* 2000; Charmantier & Blondel 2003; but see Valcu & Kempenaers 2008), suggesting that male and female contributions to laying dates could be disentangled, but also that any difference found between Muro and Pirio could not be attributed to differences in divorce rates.

FEMALE AND MALE COMPONENTS IN LAYING DATES

In both populations, laying dates did not display any additive genetic variance for males (see values of V_{Am} in Table 2). Male permanent environment effects were only significant in Pirio when no other random effect was included (Table 2, model P3) but were null in all other models. In contrast, laying dates displayed an additive genetic female component in Muro (best model based on AIC is M7 with $V_P = V_{Af} + V_R$) and Pirio (best model is P5 with $V_P = V_{PEf} + V_{Af} + V_R$), with heritabilities of 0·43 ± 0·07 and 0·20 ± 0·12 respectively. This difference in heritability between the two populations could be due to the difference in complexity of the best fitted models, since model M5 in Muro, equivalent to P5 in Pirio, provides a heritability estimate of 0·41 ± 0·23. In Pirio, permanent between-female differences (V_{PEf}), over and above those due to additive genetic effects, explained 23% of the total variance in laying dates.

Discussion

This study demonstrates that in Corsican blue tits, the timing of rapid gonadal growth in females is completely differentiated between Muro and Pirio, and is in agreement with local habitat characteristics. Opposite conclusions were previously made in males, that start their rapid gonadal maturation approximately at the same time, regardless of the habitat considered (Caro *et al.* 2006). Locally adapted breeding periods can therefore occur even when an important asynchrony

Table 2. Decomposition of variance in laying dates into male and female components in two blue tit populations (Muro, $n = 454$ breeding records from 1994–2007; Pirio, $n = 1228$ records, from 1979–2007). Variance components included residual variance (V_R), variance due to permanent environment differences between females (V_{PEf}) or males (V_{PEm}), or due to additive genetic female (V_{Af}) or male effects (V_{Am}). All models included year of breeding, age of female and age of male as fixed effects. The significance of a variance component was estimated with a likelihood-ratio test (LRT) comparing the deviance of models including/excluding this component with a χ^2 distribution (d.f. = 1): *$P < 0{\cdot}05$, **$P < 0{\cdot}0001$. The best models based on minimal AIC are highlighted in bold

Study site	Model	V_R ± SE	V_{PEf} ± SE	V_{PEm} ± SE	V_{Af} ± SE	V_{Am} ± SE	Compared with model	LRT	AIC
Muro	M1	43·8 ± 3·0	–	–	–	–			2117·0
	M2	25·8 ± 3·0	18·8 ± 3·9	–	–	–	M1	27·3**	2091·7
	M3	42·0 ± 4·2	–	2·0 ± 3·5	–	–	M1	0·3	2118·6
	M4	25·8 ± 3·0	18·8 ± 3·9	$< 10^{-6}$	–	–	M2	0	2093·7
	M5	25·6 ± 3·0	0·8 ± 10·2	–	18·6 ± 10·8	–	M2	2·3	2091·4
	M6	24·5 ± 3·5	0·7 ± 10·5	–	18·4 ± 10·8	$< 10^{-6}$	M5	0	2093·1
	M7	25·6 ± 3·0	–	–	19·4 ± 4·0	–	M1	**29·6****	**2089·4**
Pirio	P1	24·3 ± 1·0	–	–	–	–			5114·5
	P2	14·3 ± 0·8	11·0 ± 1·3	–	–	–	P1	133·8**	4982·7
	P3	22·5 ± 1·2	–	2·0 ± 0·9	–	–	P1	5·7*	5110·8
	P4	14·3 ± 0·8	11·0 ± 1·3	$< 10^{-6}$	–	–	P2	0	4984·7
	P5	14·3 ± 0·8	5·8 ± 3·4	–	5·2 ± 3·2	–	P2	**4·1***	**4980·6**
	P6	14·3 ± 0·8	5·8 ± 3·4	–	5·2 ± 3·2	$< 10^{-6}$	P5	0	4983·9
	P7	14·5 ± 0·8	–	–	10·9 ± 1·3	–	P1	133·0**	4983·5

in the final stages of gonadal maturation occurs between males and females of a single population (e.g. Pirio). In other studies investigating male and female gonadal growth, both sexes were found to be better synchronized (Wingfield & Farner 1978; Moore *et al.* 2005; Partecke *et al.* 2005). The present results also demonstrate that male early sexual development did not advance female gonadal growth in Corsican blue tits. Female therefore seem to rely on other environmental cues (or on the same cues but at a different level) than males, and their sexual development should be the limiting factor that determines the final timing of reproduction. This prevalent role of females in the determination of the differences in local population laying dates in these highly contrasted environments is reinforced by quantitative genetic analyses showing that the timing of breeding can be viewed as a sex-specific trait where females have the exclusive genetic influence.

ASYNCHRONOUS GONADAL DEVELOPMENT BETWEEN FEMALE POPULATIONS

The precise physiological mechanisms responsible for the differentiation of female follicular development remain elusive at present. Two non-mutually exclusive types of controls are conceivable based on available evidence.

In aviaries, Pirio females always display a late egg laying period, except when held under very long photoperiods (17L : 7D) (Lambrechts *et al.* 1997b), suggesting the presence of a higher photoperiodic threshold in females from Pirio compared to the other populations. Furthermore, birds from Pirio seem to be less sensitive to non-photoperiodic factors in aviaries than Muro birds. The egg laying date is indeed affected by the size of aviaries and complexity of vegetation in captive Muro birds but not in subjects originating from the Pirio population (Caro *et al.* 2007). Together, these data would suggest that the delayed egg laying in Pirio females is controlled at the brain/hypothalamic level where photoperiodic and supplemental cues are integrated (e.g. Maney *et al.* 2007) to generate the adequate increase in GnRH secretion when suitable reproductive conditions become available, in particular when the critical photoperiod is reached. According to this model, in the spring, the photoperiodically induced secretion of hypothalamic GnRH and pituitary gonadotrophins would occur several weeks later in Pirio females compared to Muro ones. The absence of high VLDL levels before the end of April in Pirio females would therefore reflect the absence of hypothalamo–pituitary stimulation of the ovary that would then not produce oestrogens, so that in turn the liver would not be stimulated to produce VLDL.

Alternatively, we cannot exclude that the two female blue tit populations have similar photoperiodic sensitivities, as suggested in males, and that the difference between populations resides elsewhere in the HPG axis, at the level of the ovary or the liver. In this case, an earlier increase in VLDL concentrations in Pirio females would have been hampered by the absence of response of the ovary to gonadotrophins, or of the liver to oestrogens. In this hypothesis, the fine control of breeding by supplemental cues would therefore lie at the gonadal/liver level, rather than in the brain, hypothalamus and pituitary. A typical example of such a gonadal control of seasonality can be found in the lack of final follicular maturation regularly observed in species held in captivity and subjected to photoperiodic experiments, even when gonadotrophin levels are significantly increased (Farner *et al.* 1966; Wingfield 1983; Silverin & Westin 1995; Ball & Balthazart 2002; Sockman *et al.* 2004). The absence of final gonadal maturation is generally limited to one sex: male testis growth is often complete while ovaries remain undeveloped under similar conditions (King *et al.* 1966; Wingfield & Farner 1978; Silverin & Westin 1995). By nature, however, these photoperiodic studies have to be conducted in captivity, an environment that is known to dramatically reduce the array of environmental cues available (Visser & Lambrechts 1999), which often modifies the concentrations of reproductive hormones (e.g. gonadotrophins) in captive birds compared to those described in the field (Farner *et al.* 1966; King *et al.* 1966; Wingfield 1983; Dawson & Goldsmith 1984; Dawson 1997). Therefore, the fine assessment of the role of the different levels of the HPG axis in the control of the final stages of follicular maturation may be difficult due to confounding detrimental effects of artificial environments on wild females. Baptista & Petrinovich (1986) collected nestling female white-crowned sparrows in the wild, hand-reared them and readily obtained ovulations in captivity, under conditions in which ovulation had never been reported in wild-caught adult females. They suggest that wild-caught females may imprint on their habitat characteristics early in life and later require the full range of environmental stimuli to complete egg formation when subsequently raised in captivity. Unfortunately, these observations were gathered incidental to studies on song development, and therefore no physiological measurements were undertaken. These results however, suggest that hand-reared chicks may more easily display in captivity the species-typical concentrations of GnRH and gonadotrophins and therefore allow the precise investigation of the respective roles of photoperiod and of supplemental cues in the final maturation of female HPG axis.

Whatever the mechanism involved, our results clearly demonstrate that follicular development in free-living female blue tits matches the population differences in optimal breeding time. Thus egg laying dates recorded in the field (Blondel *et al.* 1999) and in captive populations breeding in aviaries (Lambrechts *et al.* 1997b), as well as field data describing changes in plasma yolk precursor levels in females (this study) sharply contrast with the synchronous early development of the reproductive system observed in the two male populations (Caro *et al.* 2005b, 2006).

FEMALE DRIVE ADAPTIVE BREEDING DIFFERENTIATION

Using unique long-term datasets based on 14 and 29 consecutive breeding seasons in Muro and Pirio, simple phenotypic linear mixed models showed no or very little variance in laying dates between males in both populations (models M3 and P3) whereas differences between females explained around 40% of the total phenotypic variance in laying dates (models M2 and P2). Extending these models to quantitative genetic animal models showed that the female component could be partly attributed to additive genetic effects, with estimated heritabilities of 0·43 ± 0·07 and 0·20 ± 0·12 in Muro and Pirio. These results thus demonstrate that the egg laying date can be considered as a sex-specific trait for which the variance is not explained by male influences, apart from the noticeable influence of male age, as noticed before in birds (e.g. van de Pol *et al.* 2006). Because local laying dates are adaptive in both Muro and Pirio populations (Blondel *et al.* 1999), this means that females alone determine the local optimal breeding time and may have driven the rapid adaptive evolutionary differentiation observed between the two populations studied (see Blondel *et al.* 1999). These results are in agreement with earlier studies showing no influence of male's identity on a pair's laying date and clutch size in collared flycatchers (*Ficedula albicollis*, Sheldon *et al.* 2003) or oystercatchers (*Haematopus ostralegus*, van de Pol *et al.* 2006). It is also along the line of previously documented higher repeatability and/or heritability of egg laying dates in female great tits (*Parus major*) compared to males (van Noordwijk *et al.* 1981; van der Jeugd & McCleery 2002; Gienapp *et al.* 2006; but see Newton & Marquiss 1984). Only in one population of birds, a colony of mute swans *Cygnus olor*, did male identity statistically influence breeding dates (Charmantier *et al.* 2006). To our knowledge, this is, however, the first set of data suggesting that the sex difference classically highlighted plays a decisive role in the breeding differentiation of local populations, and that this sex difference can be directly correlated to physiological measurements of the seasonal development of the reproductive system. We therefore demonstrate that a female bird population is able to match its reproduction with the local optimal breeding period while males, not only start their physiological development too early, but also breed less reproducibly than females and play no role in the genetic determination of this optimal laying period.

Egg laying dates in the Corsican blue tit populations under study showed heritability values comparable to results described in other studies (usually between 0·16 ± 0·07 and 0·41 ± 0·08, Sheldon *et al.* 2003). Higher sample sizes in Pirio conferred sufficient power to decipher between female effects attributable to permanent environmental differences on the one hand, such as non-additive genetic effects (e.g. dominance) or early-growth environment, and to additive genetic effects on the other hand. This decomposition partly explains the somewhat low laying date heritability in Pirio. However, it could also be related to heritability decreasing in constraining as compared to favourable conditions as observed in different taxa in the wild (see reviews in Hoffmann & Merila 1999; Charmantier & Garant 2005). In the Mediterranean region, different studies have suggested higher constraints in evergreen compared to deciduous habitats, notably in terms of food availability and parasitic loads (Zandt *et al.* 1990; Blondel *et al.* 1993; Hurtrez-Boussès *et al.* 1998; Tremblay *et al.* 2003), that can for example significantly reduce the heritability of chick tarsus length (Charmantier *et al.* 2004a). Finally, the levels of extra-pair paternity (EPP) are very high in Pirio (25% of EPP in 68% of broods), while comparatively lower in Muro (18% of EPP in 50% of broods, Charmantier *et al.* 2004c). Based on simulations using these blue tit pedigrees, Charmantier & Reale (2005) have previously shown that such levels of misassigned paternities when conducting quantitative genetic analyses can result in underestimating heritability.

In conclusion, this study stresses the importance of considering both sexes in the investigation of bird life history traits related to reproduction. This is particularly important in the many species showing some sexual divergence in reproductive roles, as it is the case in blue tits, because the environmental cues used by males and females are expected to differ, as well as the selective pressures that act on them (Ball & Ketterson 2008). It also implies that adaptation of a population's timing of breeding to increasing temperatures will depend on microevolutionary processes occurring solely via selection on females and not males.

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