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their chicks still lay in May the following year, when kept in captivity under natural photoperiod (M.M. Lambrechts, unpublished data).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 *ed to29(s 7TjCs6s 0 0 0 scn-146.33TDet al*

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 s. Partial analyses made on data from periods when samples had been collected in both years, failed to identify any difference between the 2 years ($P > 0.05$, 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 $P \leq 0.05$.

Q AN I A I E GENE IC ANAL I

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

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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.

DI O CE   A E

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

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VLDL concentrations (Fig. 1) varied significantly during the breeding season ($F_{8,155} = 2\cdot636$, $P = 0\cdot0098$). The interaction between study sites and sampling periods was highly significant ($F_{8,155} = 5\cdot112$, $P < 0\cdot0001$) but there was no overall difference between sites ($F_{1,155} = 0\cdot645$, $P = 0\cdot4230$). 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 : $F_{1,64} = 3\cdot326$, $P = 0\cdot0729$).

EGG LA ING PE IOD   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

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.

A NCH ONO GONADAL DE ELOPMEN BE EEN FEMALE POP LA ION

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 maturs, es hypothesis, the4 T

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