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Individual variation in avian reproductive physiology does not reliably predict variation in laying date

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ABSTRACT

Most animals reproduce seasonally. They time their reproduction in response to environmental cues, like increasing photoperiod and temperature, which are predictive for the time of high food availability. Although individuals of a population use the same cues, they vary in their onset of reproduction, with some animals reproducing consistently early or late. In avian research, timing of reproduction often refers to the laying date of the first egg, which is a key determinant of fitness. Experiments measuring temporal patterns of reproductive hormone concentrations or gonadal size under controlled conditions in response to a cue commonly assume that these proxies are indicative of the timing of egg laying. This assumption often remains untested, with few studies reporting both reproductive development and the onset of laying. We kept in total 144 pairs of great tits (*Parus major*) in separate climate-controlled aviaries over 4 years to correlate pre-breeding plasma luteinizing hormone (LH), prolactin (PRL) and gonadal growth with the timing of laying. Individuals varied consistently in hormone concentrations over spring, but this was not directly related to the timing of gonadal growth, nor with the laying date of the first egg. The timing of gonadal development in both sexes was similarly not correlated with the timing of laying. This demonstrates the female's ability to adjust the onset of laying to environmental conditions irrespective of substantial differences in pre-laying development. We conclude that stages of reproductive development are regulated by different cues, and therefore egg laying dates need to be studied to measure the influences of environmental cues on timing of seasonal reproduction.

1. Introduction

Seasonal timing of reproduction is a key life-history trait with a large impact on reproductive output. A mismatch between reproduction and seasonal high food abundance leads to fewer surviving, and lower quality offspring, or lower winter survival of the parents [7,24,32,39,40,42]. In avian research, timing of reproduction often refers to the laying date of the first egg in spring [41]. However, the initiation of gonadal growth and the underlying activation of the reproductive endocrine system is also part of the timing mechanism [5,8,13,14,17,20,23,34,37]. This dual vision originates from the fact that evolutionary ecologists are more concerned with behavioral decisions and their fitness consequences, while physiologists are by definition more interested in the proximate mechanisms underlying a certain phenotype, such as gonadal growth and ovulation. Experimental studies combining ecological and physiological approaches to the timing of reproduction have increased understanding of this life history trait [e.g. 4,5,27,43,46] and are thus especially valuable.

In temperate zone birds, the actual process of egg laying is preceded by a physiological cascade mediated by neuroendocrine responses to environmental cues. Egg laying is preceded by the (re-) activation of the hypothalamo-pituitary-gonadal axis by short photoperiods during fall causing the dissipation of photorefractoriness and increased GnRH-I gene expression [36]. During winter and early spring the increase in day length stimulates increased secretion of GnRH-I, leading to a release of luteinizing and follicle stimulating hormone (LH and FSH) from the pituitary and a period of gonadal development that lasts several weeks. LH and FSH act synergistically to facilitate gonadal maturation and spermatogenesis: at the level of the gonads FSH affects Sertoli cell function in males and granulosa cell function in females and stimulates growth of immature follicles in the ovary. LH affects Leydig cell function and stimulates the secretion of androgens in males, while an acute surge in LH triggers ovulation in females. These photoinduced processes, culminating in the laying of the first egg, are fine-tuned by supplementary cues, including temperature, and possibly

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other climatic and phenological cues, including the seasonality of prey items [9,47,51].

Due to the difficulties in measuring laying dates in captivity in response to a likely cue, manipulative experiments make use of proxies that are presumed to indicate the timing of egg laying, but are also studied for their own sake e.g. [11,15,18,30,33,38,48–50]. Ideally for getting independent data points, pairs of birds would be kept in isolated aviaries, in which environmental variables can be individually regulated. However, this is often not feasible and in many manipulative experiments, the shortcut of examining reproductive physiology instead of a laying date allows for a larger sample size, e.g. many animals (of only one sex) per room or cage, as well as for shorter and less complex experimental designs, as the laying stage does not have to be reached. The most widely used proxies in avian research are, on one hand, gonadal growth, which means the increase in volume of the male left testis, or, more rarely [2] the development of the largest follicle in the female ovary, as well as plasma concentrations of gonadotropins, prolactin, or sex steroids, measured either in the blood or in feces. These measures can be taken at regular intervals during different reproductive stages. More recently, also processes higher upstream in the hypothalamo-pituitary–gonadal (HPG) axis have been added to the physiologist's toolbox, including the release of GnRH-I [20,36], or even gene expression [19,22]. Emphasis has been placed on photic cues, which determine a broad window for egg laying [10,29], whereas the influence of supplementary cues has been largely neglected. Conversely, interest in processes closely associated with late reproductive stages, such as the exponential growth phase of the follicle, is increasing, using yolk precursors such as vitellogenin or very low density lipoproteins as proxies [6,26]. This avenue also investigates supplementary cues that might be taken into account in the last days before the actual egg laying takes place.

In studies concentrating on the regulation of the reproductive development by its own means, observations should be made in the context of their adaptive value, most importantly relating to the optimal timing of laying. The way in which an individual female responds to environmental cues affects selection pressures acting on both reproductive physiology, as well as timing of laying [41]. Evolution therefore optimizes both the systems of physiological regulation themselves, as well as the behavioral traits that they precede. For example, birds presumably regress their gonads outside the breeding season, because flying with heavier body weights year-round is costly and thus selected against. This makes a phase of gonadal growth in early spring necessary. Also, even though early laying is generally advantageous, as it results in more surviving offspring in that particular year, advancing the physiological development early in spring when food availability is low may impede fitness costs that counterweight these advantages.

The responsiveness to cues might change over developmental stages. It is convenient to assume that a cue, like temperature, which advances the underlying hormonal and gonadal development would also advance egg laying. Indeed, it has often been postulated that temperature influences the timing of reproduction because of an effect on the gonadal development [8,48]. However, Schaper et al. [27] showed that in climate-controlled aviaries, moderate spring temperature patterns influenced laying dates of great tits (*Parus major*) without affecting the timing of gonadal growth or increase in LH concentration.

The assumption that an early rise in gonadotropins would directly translate to early gonadal development, which again would lead to an early onset of laying, has, to our knowledge, never been explicitly tested under controlled conditions. This is basically due to the fact that few experimental studies that report laying dates also measure reproductive physiology, and studies that evaluate reproductive development seldom keep pairs of birds to obtain independent laying dates. In addition, individual variation in physiological measurements is seldom explored in detail, as physiologists mostly report mean values per treatment group in response to environmental stimuli [45].

The aim of this study was to use breeding pairs of great tits to investigate if the relationship between the timing of individual early reproductive development and egg laying is as tight as assumed, or alternatively regulated by different processes, resulting in substantial variation in the interval between, for instance, full gonadal development and laying date. Although the prime objective of the experiments presented here was to show the influence of temperature cues on avian physiology and the onset of laying, the setup allows us to relate the timing of the individual rise in LH, PRL, as well as the growth of testes and ovarian follicles to laying date. This study does not include measurements of late stages of the reproductive maturation, such as yolk precursors. These changes, which are connected to an increase in estradiol following gonadal maturation, are tightly correlated with the laying date decision and most likely happen during the last days pre-laying. In the current setup we cannot comment on the feasability of using these measures as proxies. We were interested in physiological mechanisms that determine the individual variation in the onset of laying in response to environmental cues perceived well in advance of the laying date, which in wild great tits varies by up to one month between individual females and can therefore not be significantly regulated by differences in late reproductive maturation.

If predictive supplementary cues affect reproductive physiology, and consequently egg laying via early reproductive development in early spring, we expect a relationship between the timing of a rise in LH, gonadal development and laying date. In contrast, if physiological processes are fine-tuned by different cues, we expect only a loose relationship between these reproductive components and the timing of laying. In addition, it was suggested that variation in pre-laying PRL titers was associated with laying dates in house sparrows, *Passer domesticus* [21] and with egg laying rate in chicken, *Gallus gallus domesticus*, and thus would play a stimulatory role in gonadal development [16], We therefore tested for a correlation between plasma PRL concentrations pre-laying and laying dates.

2. Materials and methods

2.1. Birds

This study used 144 first-year breeding pairs of great tits spread over four years. Birds were offspring of known wild parents at the Hoge Veluwe National Park (The Netherlands), and were taken to captivity as complete broods in 2006–2009, respectively. On day 10 post-hatching, chicks were taken to the Netherlands Institute of Ecology (Heteren) for hand-raising [12].

After independence, fledglings were transferred to single-sex groups in open outdoor aviaries ($2 \times 4 \times 2.5$ m), where they were housed until December. Breeding pairs were formed randomly, avoiding sib-matings. Due to fatalities in the young birds, we formed some pairs by using 29 additional spare birds over 4 years, which were hand-raised in the same fashion. On the 1st of December the pairs were placed in climate-controlled aviaries to breed in the next year.

2.2. Aviaries

Breeding pairs were housed in 36 separate indoor aviaries ($2 \times 2 \times 2.25$ m) under a light regime mimicking the natural photoperiod, which was adapted twice weekly (i.e. for 52°N increasing from 7.45L:16.15D at the winter solstice to 16.30L:7.30D at the