Most songbirds learn their songs from adult tutors, who can be their father or other male conspecifics. However, the variables that control song learning in a natural social context are largely unknown. We investigated whether the time of hatching of male domesticated canaries has an impact on their song development and on the neuroendocrine parameters of the song control system. Average age difference between early- and late-hatched males was 50 days with a maximum of 90 days. Song activity of adult tutor males decreased significantly during the breeding season. While early-hatched males were exposed to tutor songs for on average the first 99 days, late-hatched peers heard adult song only during the first 48 days of life. Remarkably, although hatching late in the season negatively affected body condition, no differences between both groups of males were found in song characteristics either in autumn or in the following spring. Similarly, hatching date had no effect on song nucleus size and circulating testosterone levels. Our data suggest that late-hatched males must have undergone accelerated song development. Furthermore, the limited tutor song exposure did not affect adult song organization and song performance.

1. Introduction

It is well established that learning plays an important role in the development of the complex songs of oscine songbirds [1,2]. Generally, in young songbirds, two phases of song learning are recognized: an auditory phase, during which songs are memorized, followed by a motor phase where young birds change their vocal output until it matches their auditory template [2]. After a phase of plastic song whose duration varies across species, young birds crystallize their songs towards their first breeding season [2]. The sensitive phase for song memorization typically starts around fledging and ends well before juvenile moult in summer. However, the process of template formation is still not well understood. The amount of tutor song exposure that is necessary varies between species but as little as a few hours have been found to be sufficient [3–7]. As breeding seasons can be long, and the vocal activity of adult conspecifics changes, the amount of adult song a juvenile is exposed to during its sensitive period can vary considerably depending on the time of hatching.

Domesticated canaries, similar to their wild ancestors, have an extended breeding season lasting four to five months [8]. Thus, within the same season, juveniles can differ in age by up to four months. The aim of this study was to investigate, for the first time, whether this age difference and the associated difference in the presence of adult singing males as potential tutors is reflected in juveniles’ song development, their adult song characteristics and/or song system morphology.

2. Material and methods

Full methods are provided in the electronic supplementary material.

(a) Animals

Domesticated canaries (40 males, 24 females) were kept in an established colony in Seewiesen/Germany in a large aviary with inside and outside compartments under natural day length. In 2011, the breeding season lasted from the end of March to the beginning of
analyses on 20 songs per bird. The following parameters were based on this observation, we performed detailed song cumulative curve between the number of songs and repertoire day of the respective recording session. Birds were sacrificed after the last recording session in spring. (Online version in colour.)

**Figure 1.** Diagram showing the timeline of song learning for early-hatched (a) and late-hatched males (b). Blood samples from each bird were taken on the last day of the respective recording session. Birds were sacrificed after the last recording session in spring. (Online version in colour.)

August. In total, 40 fledglings were raised, of which one-third were males. Mean number of males per nest was 0.9 ± 0.7. For the experiment, 11 juvenile males from different nests were available. Hatching dates of early-hatched males (N = 5) were between 11 April and 18 May 2011 and of late-hatched males (N = 6) between 2 June and 10 July 2011. Blood samples were taken in autumn 2011 between 19 October and 12 December and in spring 2012 between 27 March and 6 May. All males were sacrificed in spring 2012.

**b) Song activity measurements**

Song activity of adult tutor males in the aviary was estimated as the number of songs heard within a 5-min-period, which was randomly chosen within three time periods per day (morning, noon and afternoon). These measurements were taken at 10-day intervals throughout the breeding season in 2011 from 8 April to 1 August. Offspring song activity was measured in the aviary in autumn 2011 between 13 October and 5 January and in spring 2012 between 1 February and 27 March. For each male offspring, the average of the number of songs produced within three 5-min periods during the morning on 5 consecutive days was recorded.

**c) Song recording and analysis**

Juveniles were recorded in autumn 2011 between 19 October and 5 January and in spring 2012 between 1 February and 27 March, and therefore consider the calculated power sufficient.

**f) Statistical analysis**

We employed a REML-model using JMP software with birth date and season as fixed factors and bird ID as random factor. Morphological data and age were analysed using unpaired t-tests. Differences with p-values less than 0.05 are termed as significant. Statistical power was estimated by first determining a correlative value among repeated measures on a previously published dataset on seasonality of song parameters in the same canary population where the effects were large [9] using JMP. This value was then entered into a power analysis using G*Power 3.1 [11], specifying, within ‘f-test’ a repeated measures model. Together with an expected (large) effect size of 0.45, this resulted in an actual statistical power of over 0.8 for our dataset. Based on these calculations, we expect large effects in our song variables and therefore consider the calculated power sufficient.

**3. Results**

**(a) Song activity of tutor males**

Early-hatched males heard adult song on average for the first 99 ± 15 days (range: 74–111) and late-hatched males for 48 ± 15 days (range: 21–59) of life (figure 1). Song activity of adult males decreased as the breeding season progressed ($F_{3,35} = 6.68$, $p = 0.014$, figure 2). Late-hatched juveniles were exposed to significantly smaller amounts of song compared with early-hatched peers at all developmental stages, i.e. hatching (~60%), fledging (18 days of age, ~64%), independence (30 days of age, ~72%) and at 45 days of age (~86%). Adult singing commenced again after moult in autumn (2 October).

**(b) Age difference of juveniles**

The songs of early- and late-hatched males were recorded in their first autumn and in the following spring when birds were adult. At the respective times of recording, late-hatched birds were on average 45–54 days younger than early-hatched birds (autumn: $t = 3.52$, $p = 0.014$, spring: $t = 4.72$, $p = 0.001$).

**(c) Effect of hatching date on song development and adult song**

Both groups of juvenile males sang plastic song during their first autumn and crystallized song during their first breeding season (from mid-January on). At both times, song activity did not differ between groups. Further, early- and late-hatched...
males did not differ in any song parameter measured either in autumn or in spring (table 1). Variances were significantly different between both groups for the proportion of single syllables in spring ($F_{1,9} = 10.26, p < 0.011$), indicating that late-hatched birds were more similar to each other regarding this parameter than early-hatched birds (Levene’s test).

(d) Effect of hatching date on seasonality of song and testosterone levels

Hatching date did not influence seasonal changes in song characteristics and plasma testosterone levels. Both groups showed higher song activity in spring with males singing longer songs that contained fewer single syllables (table 1). Plasma testosterone levels were higher in spring compared with autumn with no group differences (table 1).

(e) Effect of hatching date on body mass and song system morphology

Males did not differ in the volume of song control nucleus HVC in their first breeding season ($t = 0.523; \text{d.f.} = 8; p = 0.615$). However, at time of sacrifice, early-hatched birds were heavier than late-hatched birds ($t = 2.925; \text{d.f.} = 8; p = 0.019$).

4. Discussion

Our study reveals two interesting findings. First, hatching late in the season does not impair song development in juvenile male canaries. Second, reduced tutor song exposure has no adverse effects on song quality.

Juveniles differed in age by up to three months, and at both recording times late-hatched males were on average 50 days younger than early-hatched males. However, as we found no group differences in song characteristics, neither in autumn nor in the following spring, both groups of males were at similar stages in their song development. This implies that late-hatched males underwent accelerated sensorimotor learning compared to early-hatched males. Normally, canaries develop plastic song with about two months of age, and full song is crystallized when seven to eight months old [12]. Song organization changes significantly during this period [13]. Our results show that males are flexible in their requirement of vocal motor practice, and a shortening by 50 days does not negatively affect song development. Experimental evidence from zebra finches suggests the existence of a sensitive period of vocal motor practice during the stage of sensorimotor learning [14,15]. Vocal paralysis for two to three weeks during subsong and plastic song has no lasting effects on adult song, while the same treatment towards the end of the sensorimotor phase and around the time of song crystallization leads to large song abnormalities [14]. Similarly, nightingales (Luscinia megarhynchos), open-ended learners as canaries, acquire new song types very rapidly (within 2 days) when presented during late plastic song and shortly before song crystallization [16]. These data together with our findings suggest that the advanced stages of the sensorimotor phase are important for normal song development and prolonged periods of vocal practice are not necessary. Further experiments are needed to confirm this hypothesis.

Song learning in canaries starts after weaning (post-hatching day 30–40), and learning prior to weaning has no long-lasting effect [17,18]. Late-hatched males in our study had dramatically reduced adult tutor song exposure when song learning commenced. In parasitic cowbirds, for example, where juveniles are more constrained from hearing adult conspecific song, a field study reported a negative impact of late-hatching dates on yearling song repertoires [19]. However, laboratory studies of other species suggest that actually very little tutor song exposure is necessary for template acquisition [3–6]. Nightingales learn 21 song types when presented once per day on 5 consecutive days [4]. Thus, possibly late-hatched

**Figure 2.** Song activity of adult males decreases during the course of the breeding season.

**Table 1.** Linear mixed model results for the effect of birth (early versus late) and season (autumn versus spring) on song characteristics and plasma testosterone levels. Numbers in italic denote significant results.

<table>
<thead>
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<th></th>
<th>F</th>
<th>d.f.</th>
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males still heard enough adult tutors when their song-learning process started in order to develop normal song. Moreover, songs heard from adult tutors just before song crystallization could have had lasting effects similar to the findings in nightingales [16]. As our experimental design did not allow for assessing song imitation directly, it is possible that the two groups of males differed in the degree of copying fidelity. We also cannot exclude that late-hatched males learned from their early-hatched peers as all juveniles were kept together in the aviary. Such horizontal learning was reported for canaries and other species [10, 20, 21].

Early- and late-hatched males did not differ in song nucleus size and circulating testosterone levels, which suggests that neuroendocrine factors were not responsible for the observed similarity in song characteristics. Testosterone is known to accelerate song development in canaries [18]. However, testosterone levels were low in both groups of males in autumn and they are thought to persist at such low levels for a rather long period, from juvenile moult up to 300 days of age [22]. Further, the age difference and the difference in vocal motor practice between both groups of males was not reflected in the gross morphology of song nucleus HVC confirming previous results in this species [10]. Whether other factors, such as hormone-mediated maternal effects, differentially influenced early- and late-hatched males remains to be seen. In canaries, yolk testosterone levels change with the progress of the breeding season [23]. Taken together, these data suggest that juveniles are highly flexible in the timing of their song learning programmes in response to environmental stimuli. Furthermore, an extended period of vocal practice is not a prerequisite for normal song development.

References


Ethics. Experimental procedures were approved by the Government of Upper Bavaria/Germany (reference number Az.55.21-1-54-2352-181-12). Data accessibility. Data are available at Dryad: http://dx.doi.org/10.5061/dryad.tn86f

Authors’ contributions. S.L. conceived and designed the experiment, performed the experiment and wrote the revised version of the manuscript. C.V. conceived and designed the experiment and wrote the revised version of the manuscript. J.T. performed the experiment, analysed the data and wrote the first draft of the manuscript. A.T. analysed and interpreted the data and critically revised the manuscript. All authors gave final approval for publication.

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