

Hatching late in the season requires flexibility in the timing of song learning

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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 organisation and song performance.

Keywords: vocal learning, song exposure, testosterone, HVC, canary

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 crystallise 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 4-5 months [8]. Thus, within the same season juveniles can differ in age by up to four months. This study aimed at investigating, 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. Materials and Methods

Full methods are 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 compartment under natural day length. In 2011, the breeding season lasted from the end of March to the beginning of 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 five minute-period, which was randomly chosen within three time periods per day (morning, noon, afternoon). These measurements were taken at ten 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 five minute periods during the morning on five 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 1 May in separate cages (124x40x40 cm) in a soundproofed room. This was necessary because the aviary background noise prevented high quality recordings. Birds stayed in the room until we had enough recordings. Outside the recording sessions, all juveniles remained together in the aviary. Recordings were made with a *Zoom H2* digital recorder and a *Sennheiser ME67* directional microphone. Song analysis was done using *RavenPro 1.4* software. We plotted a cumulative curve between the number of songs and repertoire size, which reached a plateau in each male after maximal 20 songs. Based on this observation we performed detailed song analyses on 20 songs per bird. The following

parameters were measured as described previously [9]: repertoire size, song length, proportion of single syllables, and syllable repetition rate (Hz).

(d) Hormone analysis

Testosterone concentration was measured by radioimmunoassay as described before [8] with a detection limit of 4.0pg/ml. Mean recovery was $91\pm 5\%$ and the intra-assay CV was 4.2.

(e) Neuroanatomical analysis

Birds were killed by decapitation between March 27 and May 8, their brains were removed and stored at -80°C . Brains were processed and analysed according to previously described procedures [10].

(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 <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

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 (Fig. 1). Song activity of adult males decreased as the breeding season progressed ($F_{3,35}=6.68$, $p=0.014$, Fig. 2). Late hatched

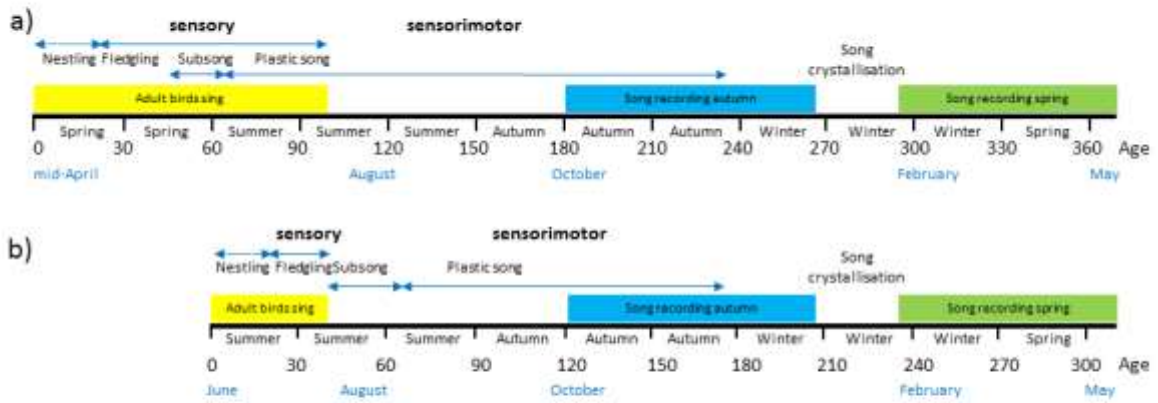


Figure 1: Diagram showing the timeline of song learning for early hatched (a) and late hatched males (b). Blood samples from each bird were taken on the last day of the respective recording session. Birds were sacrificed after the last recording session in spring.

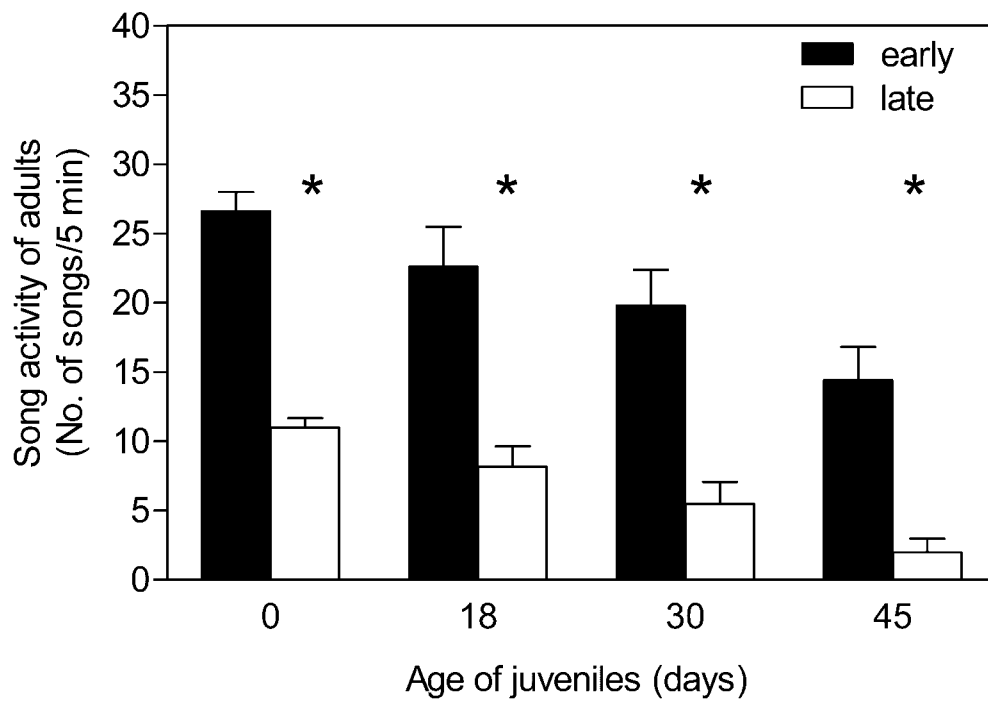


Figure 2: Song activity of adult males decreases during the course of the breeding season.

juveniles were exposed to significantly smaller amounts of song compared to 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 (October 2).

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: 160 ± 8 days vs. 205 ± 10 days; $t=3.52$, $p=0.007$, spring: 297 ± 6 days vs. 351 ± 10 days; $t=4.72$, $p=0.001$).

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).

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 to autumn with no group differences (Table 1).

Table 1: Linear mixed model results for the effect of birth (early vs. late) and season (autumn vs. spring) on song characteristics and plasma testosterone levels.

	<i>F</i>	<i>df</i>	<i>P</i>
Song activity			
Birth	0.008	1, 9	0.931
Season	8.60	1, 9	0.017
Birth x Season	0.84	1, 9	0.383
Repertoire size			
Birth	1.59	1, 8.4	0.242
Season	2.03	1, 8.2	0.191
Birth x Season	2.01	1, 8.2	0.193
Repetition rate			
Birth	0.80	1, 7.8	0.399
Season	1.05	1, 7.5	0.338
Birth x Season	3.85	1, 7.5	0.088
Single syllables			
Birth	1.39	1, 7.0	0.276
Season	11.01	1, 6.5	0.014
Birth x Season	2.19	1, 6.5	0.185
Song length			
Birth	0.06	1, 9.37	0.808
Season	10.62	1, 8.86	0.010
Birth x Season	1.13	1, 8.86	0.315
T levels			
Birth	0.88	1, 8.7	0.374
Season	44.20	1, 8.7	0.0001
Birth x Season	1.15	1, 8.7	0.313

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 ($t=0.523$; $df=8$; $p=0.615$). However, at time of sacrifice early hatched birds were heavier than late hatched birds ($t=2.925$; $df=8$; $p=0.019$).

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 2 months of age, and full song is crystallized when 7-8 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 2-3 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 two 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 (posthatching 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 reports 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 five consecutive days [4]. Thus, possibly late hatched 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, the present data suggest that juveniles are highly flexible in the timing of their song learning programs in response to environmental stimuli. Furthermore, an extended period of vocal practice is not a prerequisite for normal song development.

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Data accessibility

Data are available at Dryad: doi:10.5061/dryad.tn86f

Author contributions

S.L. and C.V. conceived and designed the experiment. J.T. and S.L. performed the experiment. S.L., J.T. and A.T analysed the data. S.L. and C.V. wrote the paper

Competing interests

We declare no competing interests.

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