

The role of sex steroid in two avian song behaviours differing in ontogenetic process

M. Ikeda, H.-A. Takeuchi^a and K. Aoki

Life Science Institute, Sophia University, 7-1 Kioicho, Chiyoda-Ku, Tokyo 102 (Japan),

and ^aDepartment of Biology, Faculty of Science, Shizuoka University, 836 Ohya, Shizuoka 422 (Japan)

Received 17 February 1994; accepted 23 June 1994

Abstract. The male Bengalese finch, *Lonchura striata*, has two types of song behaviour (directed song, DS and undirected song, US). DS and US share a basically identical syllable repertoire, sequence pattern and tempo, but differ in the time course of appearance during the maturational process. In order to examine whether this results from a difference in testosterone (T) dependency, we studied developmental changes in the fecal T level and the amounts of DS and US during the 2–4 month period (N = 7). DS appeared between 83 and 94 days of age, 4–16 days after a rise in the fecal T level. In contrast, US appeared earlier and at high frequency even when T was still at a very low level. These results suggest that DS is more dependent on the T level than US, and is not activated until the T level rises during the maturational process.

Key words. Bengalese finch; birdsong; directed song; undirected song; maturational process; fecal steroid hormone; testosterone dependency.

Song birds of the family Estrildidae have two types of song behaviour: female-directed courtship song behaviour (DS), and undirected song behaviour (US) addressing no particular object^{1,2}. In the Bengalese finch, *Lonchura striata*, although DS and US have a basically similar acoustic pattern in common (fig. 1), the two song behaviours differ in the time course of their appearance during the maturational process. Male birds begin to produce incomplete US approximately 40 days after hatching, then gradually develop US composed of a distinct structure by the age of 2 months. At this stage, however, male birds show little attention to females. They do not produce DS until 3 months, the age at which breeding can begin.

The different ontogeny of US and DS could result from a difference in testosterone (T) dependency, which may be higher in DS. We studied developmental changes in the fecal T level, and the amount of the two different song types produced by young male Bengalese finches, in order to examine whether the quantity of US or DS is temporally correlated with the T level changes.

Materials and methods

Subjects were 7 male Bengalese finches. In each bird, song counting and simultaneous fecal sampling were carried out at intervals of 3–4 days during the 2–4 month period. All birds were kept at $25 \pm 3^\circ\text{C}$ under a

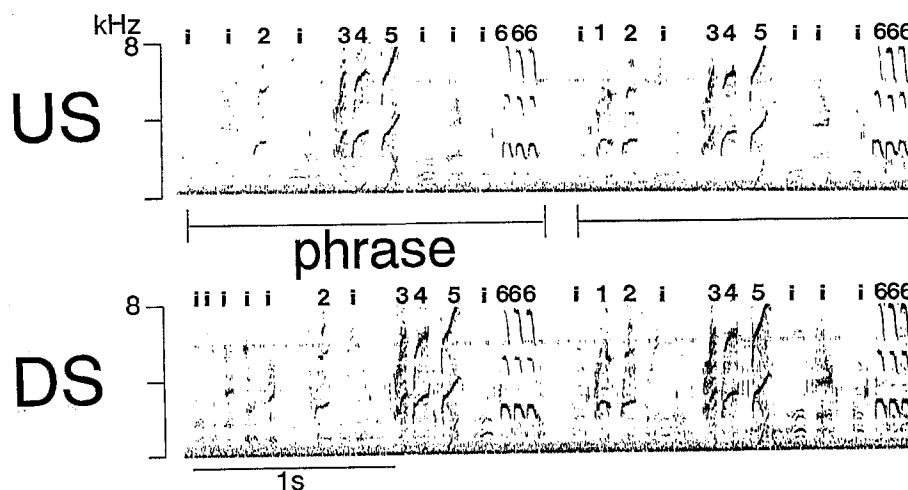


Figure 1. Sound spectrograms of a portion of undirected song (US) and directed song (DS) bouts in a male Bengalese finch, analyzed on a Kay DSP Sona-Graph model 5500. One phrase is composed of introductory notes (denoted as i) and following syllables (denoted as 1, 2, 3, ...). Typically, several phrases are repeated without interruption, constituting one bout. US and DS share a basically identical syllable repertoire, sequence pattern, and tempo (number of syllables per second).

controlled 16 h light/8 h dark cycle. The subjects were caged in groups of 2–3 birds per cage.

Song counting. In each recording session, the number of US was counted first, then, without a break, the duration of DS was measured. A subject was placed singly in a small cage (30 × 15 × 19 cm), and was brought into the acoustic chamber alone. The number of US bouts was counted for 15 min. In this study, one bout was defined as a series of successive phrases without an interval of more than 2 s. After counting US, the subject was confronted with a randomly selected female in another cage. The duration of each DS raised during the initial 5 min was measured and cumulative total recorded.

Fecal sampling. We estimated the circulating T level by measuring T in feces instead of plasma, in order to avoid unnecessary stresses due to repeated blood sampling. It has been reported that, in the Japanese quail, fecal immunoreactive gonadal steroid levels significantly correlated with plasma gonadal steroid concentrations³. Each subject was kept individually in the cage for ca. 24 h, including the time when song was counted. The whole day's feces were collected from each bird. Sampled feces were dried, weighed, and stored in a freezer until assayed.

Hormone assay. After thawing, sampled feces were extracted with distilled water and with diethyl ether. The extract was dried under air and reconstituted with 2 ml of 10 mM phosphate buffer pH7.5 containing 0.1% bovine serum albumin (Wako)⁴. Amounts of T were measured using a radioimmunoassay kit (I-125 testosterone RIA kits, Sorin Biomedica S.p.A., Italy). Assay values were calculated in per dry weight (mg) of sampled feces.

Results and discussion

It was observed that all subjects began to produce DS after a rise in the fecal T level.

Figure 2 shows typical developmental changes in DS duration, fecal T level and number of US in one subject. At 62 days old, when observation started, the male did not produce DS at all. He showed little interest in the paired female, directing his head towards her for an initial short time in the 5 min period. At 75 days, bill clattering towards the female for 3–5 s appeared, but was not accompanied by introductory notes or syllables. DS accompanied by syllables (syllabled DS) appeared at 83 days. The T level was low until 75 days, and rapidly increased at 79 days, 4 days before syllabled DS appeared. US, on the other hand, had already appeared at high frequency before 75 days, and continued afterwards.

Data of all 7 subjects are plotted with fitting curves of DS, T, US in figure 3. Syllabled DS appeared between 83 and 94 days of age, denoted day 0. The T levels were low before day-16, and then an increase occurred between day-4 and -16. For statistical comparisons, we divided

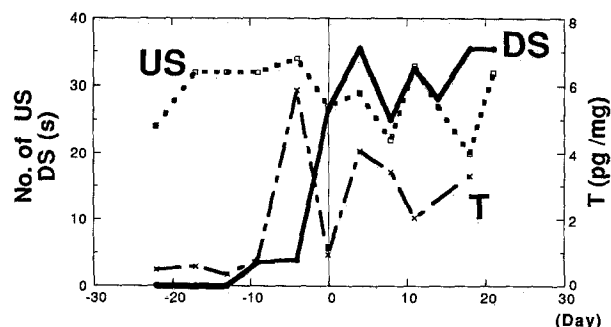


Figure 2. Typical developmental changes in fecal testosterone (T) level, duration of directed song behaviour (DS), and number of undirected song behaviour (US) in a young male Bengalese finch. Day 0 denotes the day when DS accompanied by syllables appeared, which was 83 days after hatching. The T level rapidly increased on day-4. US had already appeared at high frequency before day-10, when the T level was still very low and DS had not appeared at all.

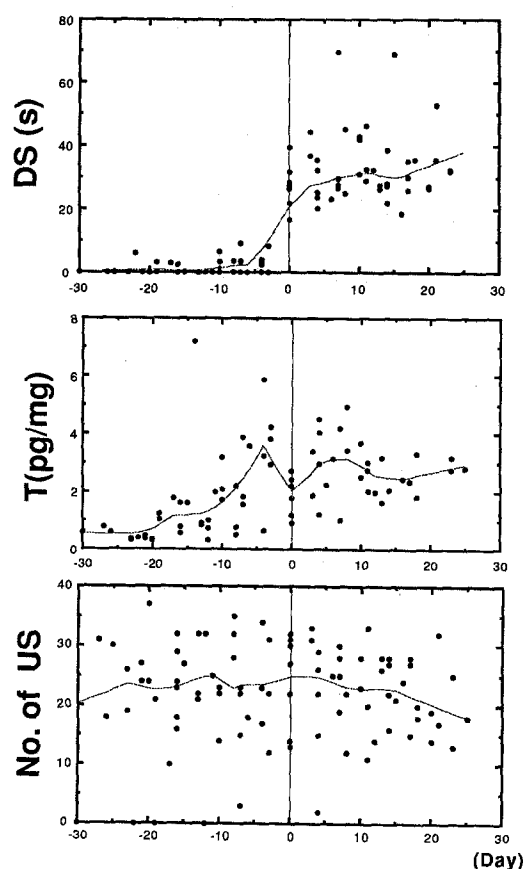


Figure 3. Developmental changes of DS, T and US summarized using data from all 7 subjects with fitting curves. Each fitting curve was obtained by a weighted fitting program (smoothing factor : 25%) of Kaleida Graph (Abelbeck Software). The tendencies coincide with figure 2. Day 0 falls between 83 and 94 days of age. The T level increase occurs between day-4 and -16. See figure 2 for abbreviations.

the period into three stages, before day-16 (early stage), between day-16 and day-4 (middle stage), and after day-4 (later stage). Maximum T values in early stage were significantly lower than those of middle and later

stage (Wilcoxon matched-pairs signed-ranks test, $p < 0.05$, two-tailed). Maximum T values of middle stage were not significantly higher than those of later stage ($p > 0.05$). US had already appeared at high frequency when T was still at a very low level, and showed small quantitative fluctuations correlated with the T level changes.

We obtained two further lines of evidence indicating that DS was more dependent on the T level than US in the mature male Bengalese finch as follows. 1) The quantities of DS, but not US, significantly correlated with the T level among individuals. 2) Castration significantly reduced the quantities of DS, but not US. The different T dependency of US and DS is in line with previous reports on the adult zebra finch (in the same family as the Bengalese finch)^{5,6}.

The present results, together with the above evidence, suggest that US is so minimally dependent on the T level as to be activated at the earlier stages even when the T level is still very low, whereas DS is not activated until the T level rises during the maturational process because of a higher T-dependency.

Walter et al. reported that song behaviours in the male zebra finch are actually dependent on oestrogenic metabolites which are derived from aromatizable androgens⁷. For the present results in the Bengalese finch, T could be metabolized to oestrogens, and the oestrogenic metabolites could then directly activate song behaviours. Further studies are in progress to analyze the effects of estradiol-17 β .

Acknowledgments. We thank Dr. H. Sakai, Nihon University, for valuable advice and kind help in facilitating radioimmunoassay, and Dr. T. Matsushima, Sophia University, for helpful suggestions. This work was supported in part by a Grant-in-Aid for Scientific Research 05680706 from the Ministry of Education, Science and Culture of Japan.

- 1 Harrison, C. J. O., *J. Orn.* 103 (1962) 369.
- 2 Sossinka, R., and Böhner, J., *Z. Tierpsychol.* 53 (1980) 123.
- 3 Bishop, C. M., and Hall, M. R., *J. Zool. Lond.* 224 (1991) 649.
- 4 Yamaguchi, N., and Ishii, S., *Zool. Sci.* 8 (1991) 1170.
- 5 Pröve, E., *J. Orn.* 115 (1974) 338.
- 6 Pröve, E., and Immelmann, K., *Horm. Behav.* 16 (1982) 121.
- 7 Walters, M. J., Collado, D., and Harding, C. F., *Anim. Behav.* 42 (1991) 445.

PRIORITY PAPERS

Manuscripts that are judged by the editors to be of high quality and immediate current interest may be given priority treatment. Publication will be within 3-4 months of receipt, providing no substantial revision is required.
