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Effect of complete and skeleton photoperiods in baya weaver, *Ploceus philippinus*

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Abstract: *A study was performed to investigate the effects of complete and skeleton photoperiods on gonadal stimulation in baya weaver, Ploceus philippinus. In August 2003, five groups of acclimatized birds (n= 7 each) were exposed to 6L: 13D: 1L: 4D, 6L:6D: 1L: 11D, 11L: 13D and 13L: 11D. Birds were refractory and their exposure to 6L:13D:1L:4D, 6L:6D:1L:11D, 11L:13D and 13L:11D did not evoke any response. This clearly indicates that the birds were insensitive to the stimulatory effects of these photoperiods and perceived them as short days. An attempt was made to test the responsivity by subjecting all these groups to long day exposure after 16 weeks of experiment. Under 16L: 8D, birds did not show a response during the first 4 weeks of exposure (week 16 to week 20), but thereafter there was a significant photoinduction except 11L:13D (week 24), but the rate and magnitude was different. Until the end of the experiment (32 weeks), all groups of birds had maximum testicular volume except 11L:13D. Birds pre-exposed to 6L:13D:1L:4D, 6L:6D:1L:11D, 11L:13D and 13L:11D responded to 16L:8D, which suggests that these photoperiods helped the birds in recovering their photosensitivity. The results from this experiment indicate that baya weaver interprets a light period depending when the light falls relative to its endogenous circadian system.*

Key words: *Photoperiod, Skeleton, Body mass, Testis, Baya weaver.*

Introduction

In the tropics, many bird species which breed during spring and summer react to photoperiod in a similar fashion to temperate species (Dittami and Gwinner, 1985; Tewary and Tripathi, 1985; Tewary and Dixit, 1986). In many species, the breeding season ends with the development of a state of refractoriness which results in spontaneous gonadal collapse and loss of response to the stimulatory effects of long day lengths (Farner *et al*., 1983; Nicholls *et al*., 1988). Once the birds are refractory to long day photostimulation, exposure for a period to short day lengths is required to render them photosensitive again (Burger, 1947; Hamner, 1964, 1968; Farner *et al*., 1983). A long day response is believed to result from the interaction of long light pulse (LLP) simultaneously with two different phases of the circadian rhythm of photoperiodic photosensitivity (CRPP). It is assumed that the beginning of the day a LLP entrains CRPP, and later in the day when extends into the photosensitive (photoinducible phase, ϕi) of CRPP it stimulates the neuroendocrine circuitry resulting in the photoperiod induced physiological responses (Follett, 1984; Kumar and Follett, 1993). This is best illustrated by the ability of two short light pulses introduced at fixed hour in the CRPP to induce the metabolic and gonadal functions (Follett, 1984; Kumar and Follett, 1993; Tewary and Kumar, 1983, 1984; Kumar, 1986, 1988). It is suggested that in a two pulse light: dark (LD) cycle paradigm (two light pulse at fixed intervals in a 24hr LD cycle), that constitutes a "skeleton" photoperiod (SKP), the first (usually longer) light pulse (main photoperiod) entrain CRPP and the second (usually shorter) light pulse falling in the night (ϕi) induces photoperiodic responses.

The aim of this study was to investigate the relative inductive effects of CP (complete photoperiod) and its corresponding SKP (skeleton photoperiod), since in both photoperiods the duration of illumination of ϕi will be similar.

Materials and Methods

The experiments were performed on adult male baya weaver (*Ploceus philippinus*). Birds were procured from local animal suppliers early in the month of August 2003. They were acclimatized to captive conditions under natural (normal) day lengths (NDL) for about a period of two weeks before they were exposed to experimental conditions. Birds under NDL were also similarly housed and kept in a room that received unrestricted environmental light and air from large windows.

The experiment was performed on 27th of August 2003 and it ran for 32 weeks (8 months). Birds were divided in five groups (n = 7 each). These groups were exposed to natural day length (NDL)-Group 1; skeleton photoperiods (6L:13D:1L:4D)-Group 2; 6L:6D:1L:11D-Group 3; and Complete photoperiods (11L:13D)-Group 4; 13L:11D-Group 5 for 16 weeks. After their exposure to the experimental schedules for 16 weeks, all the groups were transferred to long photoperiod (16L: 8D) to test further for their responsivity. Observations on body mass and testis volume were taken in the beginning and at appropriate intervals.

Birds were fed *ad libitum* on mixture of seeds of *Pennisetum* and *Setaria*. Artificial light was provided by 14 watt fluorescent tubes (CFL) at an intensity of ~500 lux at perch level and the timing of light and darkness was regulated by an automatic mueller electronic timer. The body mass was recorded using top pan balance on an accuracy of 0.1gr. The testicular size was assessed by laparotomy under local anaesthesia (Kumar *et al*., 2001). The dimensions of the left testis were recorded, and testis volume was calculated from

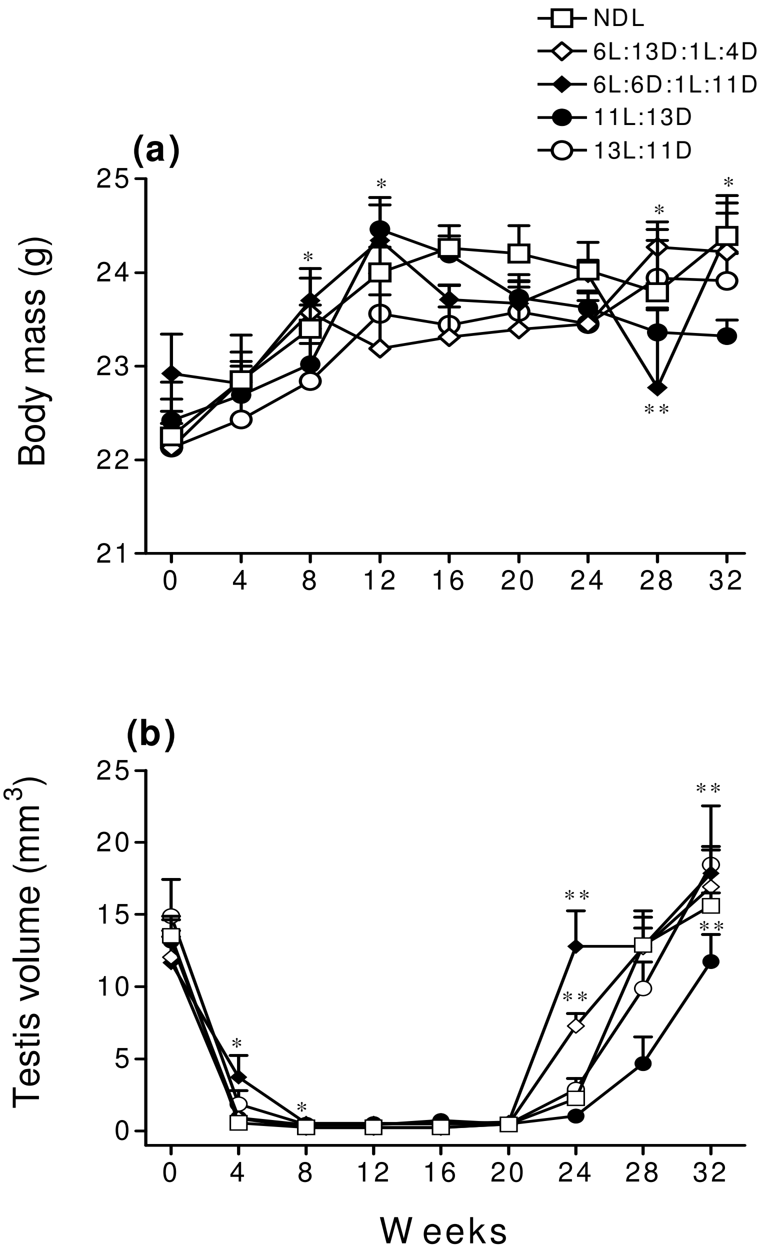

Fig. 1: Showing response in body mass (a- body weight) and testes (b- testes volume) of baya weavers (n = 7) on exposure to NDL, 6L: 6D: 1L: 11D, 6L: 13D: 1L: 4D, 11L: 13D and 13L: 11D for 16 week, and subsequently to 16L: 8D till 32 weeks. Data are plotted as Mean ± SEM. Significance of difference (p<0.05) is indicated as follows: *, difference from week 0 observation in respective photoperiods and ** difference from the value on 16 week when the birds were transferred to 16L: 8D.

$4/3\pi ab^2$, where *a* and *b* denote half of the long and short axes, respectively.

The data presented as Mean ± SEM, were analyzed by one-way analysis of variance with repeated measures (1-way RM ANOVA) followed by Student Newman-Keuls post-hoc test as well as two-way analysis of variance (2-way ANOVA) and one-way ANOVA followed by post hoc tests, if ANOVA indicated the significance of difference. Significance was taken at p <0.05.

Results

The results of this experiment show that the mean body mass among the five groups was significantly different ($F_{4,225}$=2.673, p=0.0329; 2-way ANOVA) and it was also significantly different among the weeks ($F_{8,225}$=12.81, *p*<0.0001; 2-way ANOVA). There was a differential response in different groups. Birds of NDL, 11L:13D, 13L:11D showed more significant gain and loss in mean body mass ($F_{8,40}$= 7.028, p< 0.0001, $F_{8,40}$ = 9.069, p< 0.0001, and $F_{8,40}$ = 5.729, p< 0.0001; 1-

way RM ANOVA) respectively, than the birds of 6L: 13D: 1L: 4D and 6L: 6D: 1L: 11D ($F_{8,40}$ = 3.371, p= 0.0048, and $F_{8,40}$ = 3.108, p= 0.0080; 1-way RM ANOVA) respectively (Fig. 1a).

A comparison of the testicular response of birds exposed to different photoschedules at respective observations indicated a significant difference in the effect among five treatments ($F_{4,225}$= 6.364, *p* < 0.0001; 2 - way ANOVA) (Fig. 1b). Testis were significantly regressed in all the five groups (p < 0.0001, week 0 vs 4; Newman-Keuls post hoc test), but the magnitude of decrease was different. Testes of group 3 (6L:6D:1L:11D) were not fully regressed till 4 weeks. Testes of all groups were fully regressed in 8 weeks (p < 0.001, Newman-Keuls post hoc test), and maintained same status up to the subsequent observations. The testis volume remained same in all the groups until week 16 and 20, despite the birds experiencing different photoperiods (Fig. 1b). In response to the decreasing day lengths in nature, the NDL birds, showed a significant decrease in testis volume (p < 0.001, Newman-Keuls post hoc test) till the 20 weeks (month of January) and it increased later. After exposure to long photoperiod (16L: 8D), all the groups maintained the same testis volume until next 4 weeks (20 weeks). Observations at 24 weeks, testis were significantly enlarged in all groups except 11L:13D ($F_{4,25}$= 15.38, p < 0.0001, 1-way ANOVA), but the rate and magnitude was different. Testis volume of birds of 11L:13D was very less (p< 0.05, Newman-Keuls post hoc test) in comparison to other groups. Until 32 weeks of the experiment, all groups achieved maximum testis volume except 11L: 13D.

Discussion

Day light is used as a major source of information by many vertebrate species in the regulation of their seasonal responses. In this study, which was consciously carried out during the early regressive phase of the baya's annual reproductive cycle when the sensitivity of photo-neuroendocrine system to the stimulatory effects of photoperiods would be at the lowest. This indicated that the refractoriness resulted in the form of testicular regression.

The present data (Fig. 1b) support the refractoriness on baya's photoperiodic response system. Different photoperiodic regimes exposed to complete and skeleton photoperiods, birds were refractory and their exposure to 6L:6D:1L: 11D, 6L:13D:1L:4D, 11L:13D, 13L:11D did not evoke the long day response. The timing of reproduction is based on the duration of light period and also the long photoperiod. The duration of the three light regimes 6L:6D:1L:11D, 6L:13D:1L:4D and 11L:13D is comparable to short photoperiod; 13L:11D photoperiod (long photoperiod) did not evoke the response, because birds had become photorefractory. Light pulse at zt 20 (6L:13D:1L:4D) did not evoke a long day response. These results suggest that birds enter into a state of photorefractoriness. Once the birds are refractory to long day photostimulation, exposure for a period of time to short day lengths is required to render them photosensitive again (Burger, 1947; Hamner, 1968; Farner *et al*., 1983). The rate of recovery of the responsivity to long day photostimulation, in some case, is related to shortness of short days as well as the period of treatment with short days (Turek, 1975; Nicholls and Storey, 1977). Birds under photoperiods 6L:6D:1L:11D, 6L:13D:1L:4D, 11L:13D and 13L:11D maintained regressed testes. And this clearly indicates that the birds were insensitive to the stimulatory effects of these photoperiods and perceived them as short days. An attempt was made to test their responsivity by subjecting all these groups to long day exposure. Results suggest that on transfer to the longer photoperiods birds recover their photoperiodic photosensitivity. But long day exposure of 4 weeks (till 16-20 weeks) did not evoke the response, but continuous exposure to long day photoperiod (16L: 8D) did evoke the long day response. A response in 16L: 8D by birds that were pre-exposed to 6L:6D:1L:11D, 6L:13D:1L:4D, 11L:13D and 13L:11D suggests that these photoperiods recovered photosensitivity in the refractory birds. In this experiment, 11L: 13D was given as short photoperiod, and 13L: 11D did not evoke the long day response, so it also helps to recover photosensitivity in the refractory birds. The interpretation of 6L:13D:1L:4D (given as equivalent of 20L:4D) as 6L:3D:1L: 14D (interpreted as equivalent of 10L:14D) was because of the circadian phase-dependent perception of a light period as being entraining or inducing (first) light pulse (Singh *et al*., 2002; Kumar *et al*., 2004).

The results from this study suggest that baya weaver at 29°N are quite photosensitive, and they interpret a light period and respond to it depending when it falls relative to their endogenous circadian system.

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