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Cloacal Protuberance and Body Mass in Indian Passerine Birds: Comparative Study of Quantitative Relations

Pawan Kumar [1*], Manju Bhaskar [2], Rajni Singh [3], S.K. Bhardwaj [4], Suman Tanwar [5]

[1-3] Department of Zoology, M. M. H. College, Ghaziabad. Uttar Pradesh, India

[4] Department of Zoology, Ch. Charan Singh Uni. Meerut, Uttar Pradesh, India

[5] Department of Zoology, Govt. College for Girls, Sector-14, Gurugram, Haryana, India

Corresponding Author: * Pawan Kumar

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Abstract

Many passerine species exhibit seasonal reproductive cycles, with activity restricted to a narrow vernal window. During the onset of the breeding season, male gonads undergo physiological maturation to initiate spermatogenesis. The sequestration of sperm within storage reserves induces a physical distension of the cloaca, resulting in the formation of the cloacal protuberance (CP). The objective of this study was to test the quantitative relations of these two measures and to compare their vernal temporal changes. Relative size of the cloacal protuberance (cp) was scored, providing a cloacal protuberance index (CPI: 0 to + + + +) and body mass were measured by Electric balance (with accuracy +_ 0.005Gm). The breeding phenology varies significantly between pairs in some passerines. Consequently, it is often difficult to determine an individual male's reproductive status—specifically whether he is actively producing sperm—at the time of capture. In this study of captive birds, we demonstrate that cloacal protuberance (CP) volume is significantly greater in males during their reproductively active state compared to their own inactive state. Measurements of CP volume and body mass at a single time point, therefore, directly allow the researcher to reliably determine an individual's reproductive status.

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1. INTRODUCTION

Photoperiod is an important environmental variable in determining the adaptations to the environment and providing cues from the environment to control biological functions. Photoperiodism was discovered and named in second decade of this century by W.W. Garner and H.A. Allard of the U.S. Department of Agriculture, working on the understanding of the mechanisms of timing of flowering in plants. 'Photoperiodism is the control of some aspect of life cycle by the timing of light and darkness' (Hillman, 1979)

Birds are the first vertebrates in which the role of day length (= photoperiod) was demonstrated in the control of seasonal functions. That the photoperiod could act as a temporal information date back to eighteenth century when Dutch netters realized that the song associated with breeding activity could be induced in males of several species of passerine birds by keeping them first in darkness from May to August and then returning to natural day lengths (Hoos, 1937).

William Rowan (1925) experimentally demonstrated the role of day length in gonadal development of a passerine bird species, the slate-colored junco (Junco hyemalis). In series of investigations, he established that the vernal migration and gonadal recrudescence could be induced out of season by exposure of birds in laboratory to increasing day lengths (Rowan, 1926, 1928, 1929, 1932). Since then, the role of day length in control of seasonal pre-migratory fattening, maturation and reproduction has been confirmed in many high-, mid- and low-latitude species (Murton and Westwood, 1977; Farner et al., 1983; Follett, 1984; Wingfield and Farner, 1993; Kumar, 1997; Dawson et al., 2001; Hau, 2001; Deviche and Small, 2001; Kumar et al., 2004; Rani et al., 2005; Kumar and Arvind, 2006).

Studies on reproductive cycles and other physiological factors of Indian birds at Banaras Hindu University, Varanasi (25^0N, 83^0E) were done more than five decades ago (Misra, 1948).

2. MATERIALS AND METHODS

Animals housing: This study was conducted on adult black-headed munia (*Lonchura malacca malacca*). The black-headed munia is a small tropical passerine resident breeding finch from family Estrildidae (Ali & Ripely 1974). Black-headed munia were captured in 2016, using mist nets in Meerut (290 01` N; 770 45` E), India. Birds were kept inside the photoperiodic boxes (size = 74 × 56 × 72 cm).

Experimental set up: This experiment was performed on the blackheaded munia during June month. A groups of photosensitive male birds (n = 6 per group) was exposed to short day length (9L: 15D) and constant temperature conditions (24 ± 2^oC).

After 2 weeks, all acclimatized birds grouped (by ~equal average body mass) in 7 seven groups, all these 7 groups transferred to different photoperiod illuminating experimental chambers; 10L: 14D, 11L: 13D, 12L: 12D, 13L: 11D, 14L: 10D, 15L: 9D and 16L: 8D. The Food and water changed daily two time at day time in time window (Light on 2-3 minutes and light off 2-3 minutes),

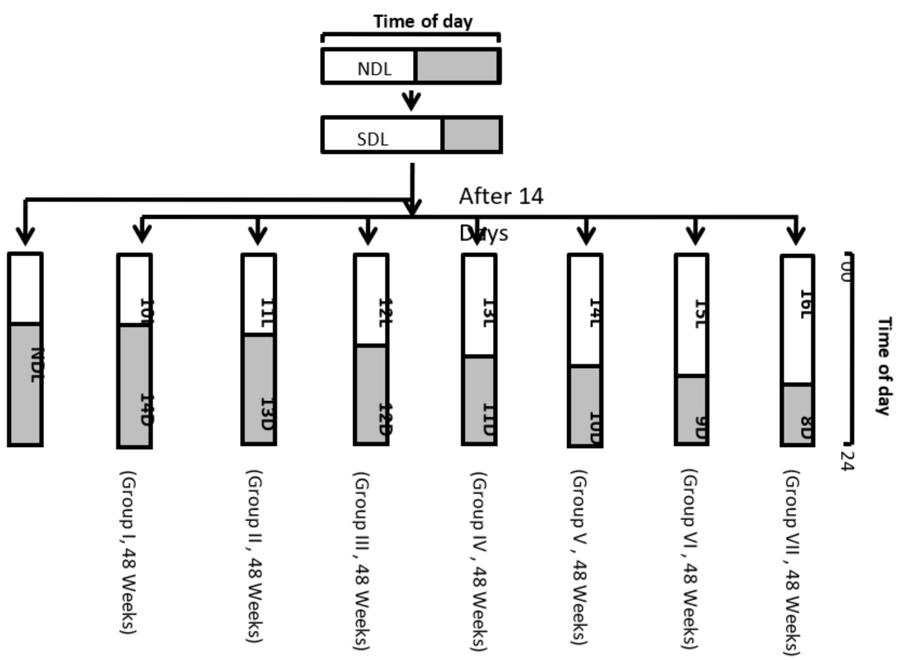

Fig. 1: Schematic representation of experimental protocol. Each bar covers 24 h period. The SDL (9L:15D) means this light illuminating period for 14 days is suitable for birds' acclimatisation, and the Different Male birds make Eight groups (Each has 6 male birds with average body weight). The 7 groups illuminate different photoperiods 10L:14D to 16L:8D and one group put in NDL for 180 Days. During light phase all experimental birds were exposed 460 lux light intensity at and dark time received dim light illumination (0.3 lux).

Data collection

Wide array of parameters as indicated in the respective experimental designs, were recorded at regular intervals. In general, observations were made on primary molt, Cloacal protuberance, body mass (body weight) and gonadal size. Data from these measurements were collected at the beginning, at end of the experiment and at appropriate intervals during the experiment.

(i) Body mass

Body mass was recorded to examine the effects of weight gain or loss. Birds were individually wrapped in a cotton bag and weighed on a top pan balance to the accuracy of 0.1g. Before weighing a bird, the weight of the cotton bag was tared to zero. According to (King and Farner, 1965; Helms *et al.*, 1967; Wade and Bartness, 1984).

(ii) Molt

The pattern of molt cycle was recorded by observations on body molt (Regeneration of whole-body feathers) and primary wing feathers (called primary molt).

a) Primary molt -The pattern of molt cycle was recorded by observations on primary wing feathers (10 to 1 called primaries) and body feathers. We followed scoring of primaries pattern as outlined by Boswell (1991), Trivedi (2004), Trivedi *et al.* (2006a) and Dixit and Sougrakpam (2013) and briefly, this score pattern was as follows:

00- Worn or old feather, 01- Missing feather (just dropped), 02- From a new feather papilla, 03- merging up to one-third growth, 04- New feather has attained two-third growth, 05- New feather grown, but still growth is incomplete

Fig.2: Diagram of experimental pattern of molt primaries) was recorded by observations on primary wing feathers (00 to 05): According the Trivedi et al. (2006a) and Dixit and Sougrakpam (2013).

(iii) Testicular volume diameter

The size of gonads was considered as an index of gonadal development and regression. The development in size of gonads over a period of days can be considered as a measure of the photoperiodic effect on the summation of gametogenic changes involving at least LH, FSH and androgens (Lofts and Murton, 1973; Lofts *et al.*, 1973; Lofts, 1975). For recording change in testicular volume (mm^3) was done by unilateral laparotomy Kumar *et al.* (2001).

(iv) Cloacal protuberance

Some animal groups, especially insects, show a remarkable range of both reproductive behavior and external sex organs associated with sperm competition (review in Smith 1984, Eberhard 1985). In birds, the ecological significance of the cloacal region has received little attention, because the avian cloaca is actually quite invariant and inconspicuous compared with other morphological features. Among species with severe sperm competition, we can predict that both reproductive behavior and the external sex organs of both sexes may evolve special features. In most (if not all) passerine males, the seasonal hypertrophy of the glomus eminales (eminal vesicles) forces the posterior wall of the cloaca to form a nodular cloacal protuberance (Salt 1954, Wolfson 1954a). It can be used as an external indicator of reproductive capability (Salt 1954, Wolfson 1954a). In contrast, little is known about the female protuberance, and the cloacal region is slightly elevated from the body wall during the breeding season (Wolfson 1954a). Therefore, the cloacal protuberances have often been used to sex various species of Passeriformes (Drost 1938, Mason 1938, McCabe 1943, Wolfson 1952). Although the function of the female protuberance remains unknown, the structure in the male is a site for the storage and maturation of sperm (Salt 1954; Wolfson 1954a, b; Lake 1981). Whether the protuberances are functionally important os still unsolved.

3. RESULTS

Results are shown in figure 3 the molt cycle of wing primaries and Cloacal protuberance in male blackheaded munia. Complete molt cycles of the feathers occurred in both SD, LD and NDL transferred male group (wing primaries, $F_{40,160}=32.76$, $P<0.0001$: 1- way RM ANOVA (figure 3 a and b). In NDL transferred group wing primaries, $F_{40,160}=69.88$, $P<0.0001$, 1- way RM ANOVA) (figure 3 a), CP (Cloacal protuberance) had decreased after the breeding season by 10.30 mm3 in all males (range –6.3–53.8 mm3; average decrease

17.8%) and by 25.0 mm3 when only copulating males were included (range 5.8–53.8 mm3; an average decrease of 54.4%). Between individuals there was overlap in CP values during and after the breeding season (all males: range while in sexually active state: 11.8–76.0 mm3; range after breeding season 11.3–43.6 mm3; copulating males: range while in sexually active state: 20.9–76.0 mm3, range after season: 15.1–43.6 mm3). Cloacal protuberance (CP), $F_{40,160}=31.74$, $P<0.0001$: 1- way RM ANOVA (figure 3 c and d). In NDL transferred group wing primaries, $F_{40,160}=69.58$, $P<0.0001$, 1- way RM ANOVA) (figure 3 c).

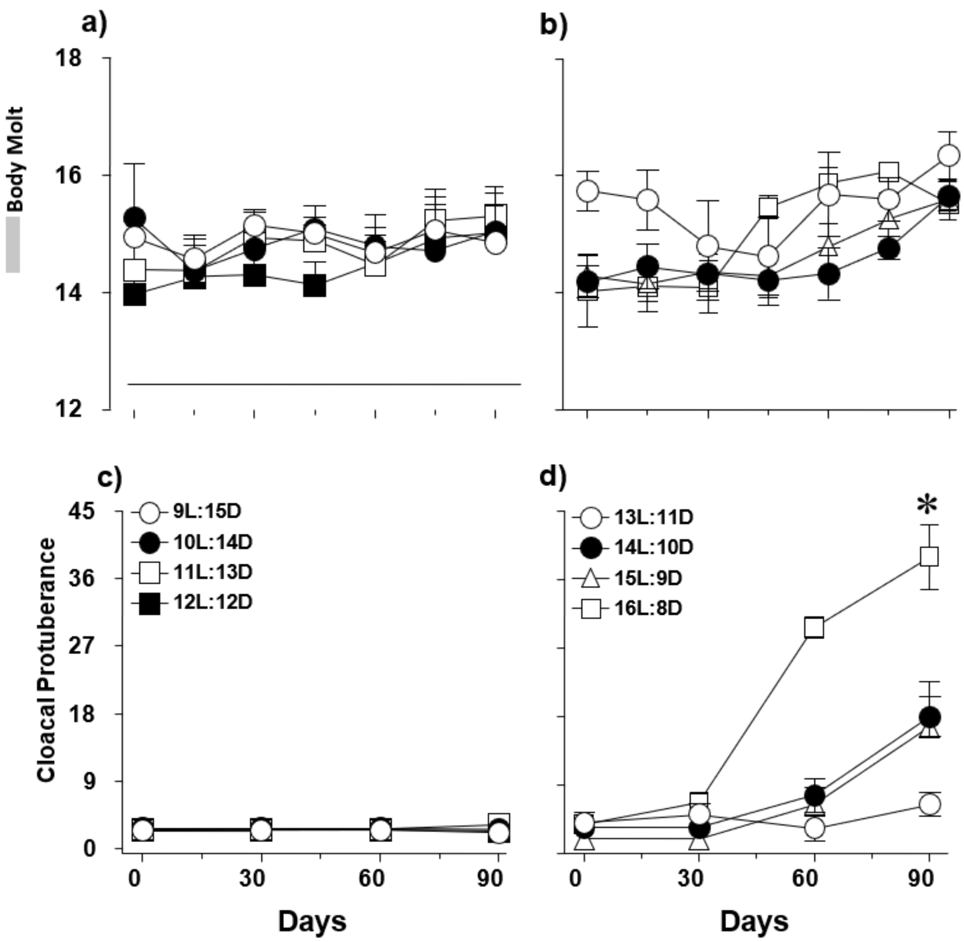

Fig. 3: Mean (±SE) body molt (a and b) and tCloacal protuberance (c and d) of the male blackheaded munia exposed to 10L:14D, 11L:13D, 12L:12D, 13L:11D, 14L:10D, 15L:9D and 16L:8D) at 460 lux intensity and one group put in NDL condition starting from (9L:15D) in month of January. Asterisks on the symbol indicate significance of difference compared to initial value (*= 16L :8D; 1-way RM ANOVA).

The Body mass and Testicular Volume are an obvious choice for the investigation of different photoperiodic responses because it was easy to investigate and because a great deal is known about their properties and functions. The Body weight in SD, $F_{40,160}=27.21$, $P<0.0001$; (figure 4a). In LD, $F_{40,160}=69.88$, $P<0.0001$, 1- way RM ANOVA) (figure 4 b). The testicular volume in SD, $F_{40,160}=32.76$, $P<0.0001$: 1- way RM ANOVA (figure 4c). While in LD, $F_{40,160}=35.70$, $P<0.0001$, 1- way RM ANOVA) (Figure 4d); and body mass of NDL, $F_{40,160}=69.88$, $P<0.0001$, 1- way RM ANOVA) (figure 4 c).

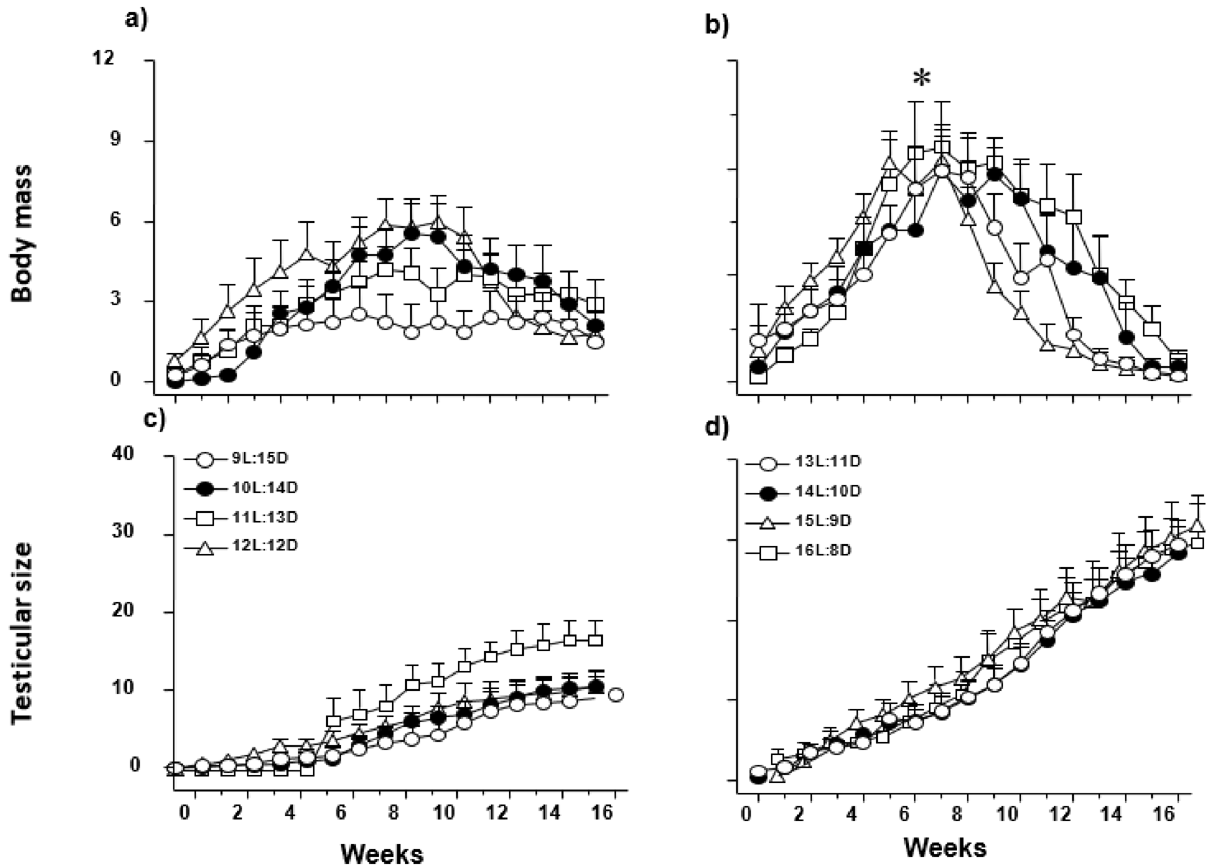

Fig. 4: Mean (±SE) body mass (a and b) and testis volume (c and d) of the male blackheaded munia exposed to 10L:14D, 11L:13D, 12L:12D, 13L:11D, 14L:10D, 15L:9D and 16L:8D at 460 lux intensity and one group put in NDL condition starting from (9L:15D) because experiment was started in month of January. Asterisks on the symbol indicate significance of difference compared to initial value (*= 16L :8D; 1-way RM ANOVA).

4. CONCLUSION

Besides body mass and molt primaries, the testicular volume and Cloacal protuberance are an obvious choice for investigation of different photoperiodic response because it was easy to collect and because a great deal is known about their properties and functions. We found that CP volume was significantly larger while males were in a reproductively active state than while they were in a non-active state. However, there was overlap in CP volume between individuals in reproductively active and non-active states. This overlap may in part be caused by the fact that during the breeding season, we measured CP on a day that copulation was observed, rather than randomly or at regular intervals during the breeding season. We may have been sampling at times when CP volume was lower than average, because males had only recently lost sperm through ejaculation. When catching a male in the field, the same bias may arise. Our findings indicate that Blackheaded munia males show visible signs of the storage of sperm in their seminal glomerae during the breeding season. However, measuring CP volume does not allow the researcher to unequivocally determine whether a wild-caught male is in a reproductively active state etc. [Salt 1954, Wolfson 1954a, Drost 1938, Mason 1938, McCabe 1943, Wolfson 1952]. To determine the correlation CP with other physiological factors different photoperiodic groups in both (LD to SD and January NDL in January) and Birds of March January showed pre-breeding phase. No any significant change in CP and physiological factors was observed, while in LD photoperiodic groups next 30 days the breeding phase became start thus the CP and molt primery, body mass and testicular volume significant change were observed in male munia. Male accentors never copulated unless females performed cloaca-presenting. The development period of a female's protuberance corresponded to the copulation period (Fig. 3 a & b). The copulation period in the renesting or second breeding attempt (i = 9.4 days) was shorter than in the first attempt (r= 29.3 days). This means that fertilization of the entire clutch potentially occurs over approximately nine days. This period is almost the same length as the presumed fertile period.

In NDL from the end of May to July, the female's protuberance was intermittently formed according to breeding attempts (Fig. 3 b). I assume that the male structure functions to store sperm, and the male is capable of successful copulations during the breeding season.

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