Photoperiod Conditions Influence Fecal Estradiol Levels in Females of Migratory Reed Bunting *Emberiza schoeniclus* and Non-migratory Meadow Bunting *Emberiza cioides*

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**Abstract.** Fecal estradiol levels in females of the migratory Reed Bunting *Emberiza schoeniclus* and non-migratory Meadow Bunting *Emberiza cioides* were measured by radioimmunoassay after extraction with ether. When the photoperiod conditions were changed stepwise from 10 hr light/14 hr darkness (LD 10 : 14) to LD 15 : 9, the fecal estradiol levels in the migratory Reed Bunting increased significantly at LD 12 : 12 and subsequently decreased at LD 14 : 10. A similar pattern of change was observed in the non-migratory Meadow Bunting. Fecal estradiol levels appear to reflect the levels circulating in plasma, indicating that estradiol levels circulating in plasma increase at LD 12 : 12 and subsequently decrease at LD 14 : 10 not only in the migratory Reed Bunting but also in the non-migratory Meadow Bunting.

**Key words:** *Emberiza cioides*, *Emberiza schoeniclus*, Estradiol, Feces, Migration, Photoperiod.

**Introduction**

Changes in hormone secretion have generally been measured by assaying the concentrations of hormone circulating in the plasma. However, little blood can be extracted from small birds, and such sampling causes great stress. Therefore, a method for measuring the levels of sex steroid hormones, such as estradiol, progesterone and androgen, in bird feces has been developed (Bishop & Hall 1991). Since changes in fecal sex steroid levels in the Japanese Quail *Coturnix coturnix japonica* show a significant positive correlation with those in plasma (Bishop & Hall 1991), the measurement of sex steroids excreted into feces makes it possible to estimate the plasma levels of these hormones in a stress-free manner.

In migratory birds, environmental factors, especially changes in daylength, influence both the hormone state (Wolfson 1945, Farner et al. 1983, Wingfield 1983) and migratory activity (Farner 1950, Farner et al. 1954, Nakamura & Ito 1982, Nakamura & Kitahara 1983). Administration of several hormones, such as prolactin, androgen and thyroid hormones, influences premigratory fattening and migratory restlessness, as evidenced by nocturnal hopping by captive birds at the time of migration (Rankin 1991, Berthold...
Thus, it appears that these hormones may be endocrine regulators of migration (Rankin 1991, Berthold 1996). However, in small migratory birds, little is known about photoperiodical effect on the hormone levels of plasma or feces.

When the photoperiod conditions are changed stepwise from 10 hr light/14 hr darkness (LD 10:14) to LD 15:9, in both sexes of the Reed Bunting Emberiza schoeniclus, which is a small migratory bird, active nocturnal restlessness appears between LD 13:11 and LD 14:10 (Nakamura & Ito 1982). On the other hand, both sexes of the Meadow Bunting Emberiza cioides, which is a non-migratory bird, do not exhibit significant changes in migratory activity under these conditions (Nakamura & Ito 1982). In addition, the fecal androgen levels in the male Reed Bunting, but not the male Meadow Bunting, increase at LD 12:12, just prior to the period of active nocturnal restlessness, and relatively high androgen levels are maintained during active restlessness (Nakamura et al. 1996).

It has been reported that implants of estradiol are ineffective in triggering vernal premigratory fattening in ovariectomized White-Crowned Sparrow Zonotrichia leucophrys gambelii (Schwabl & Farner 1989). However, ovariectomy before the vernal migration diminishes premigratory fattening (Schwabl & Farner 1989), and plasma estradiol levels increase during the migratory period (Wingfield & Farner 1978). In the present study, we examined the influence of increasing daylength on the fecal estradiol levels of females of the migratory Reed Bunting and the non-migratory Meadow Bunting.

**Materials and Methods**

Females of the Reed Bunting and the Meadow Bunting were captured in December 1993 in a field in a suburb of Kofu City (35°40' N, 130°30' E), Yamanashi Prefecture, Japan. They were maintained in individual cages (32×18×24 cm) under controlled conditions (20–24°C and 50% humidity) in LD 10:14 (lights-on at 7:00 hr) for 2 weeks and given food and water ad libitum. Thereafter, the photoperiod conditions were changed stepwise from LD 10:14 to LD 15:9; the lights-on time was advanced by 15 min/week and the light-off time was delayed by 15 min/week. Ordinary fluorescent lights were used for illumination during light periods. The Reed Bunting lives from autumn to spring in a suburb of Kofu City, migrates north in mid to late April and breeds in Hokkaido, the northern part of Japan. The birds migrate at night, as do most other small migratory birds. In contrast, the Meadow Bunting is a non-migratory bird, although some populations in the north part of Japan are short-distance migrants.

Fecal matter from 3 to 6 females was collected weekly between 10:00 hr and 15:00 hr, dried, and stored at −20°C until preparation. Samples (1.00–1.50 g) were homogenized in 4 ml of ice-cold distilled water and centrifuged at 2,500×g for 15 min at 4°C. The resulting supernatant was extracted with 4 volumes of water-saturated diethylether and the ether phase was evaporated. The dried residue was stored at −20°C until radioimmunoassay (RIA). To determine recovery of estradiol through extraction procedure, 4,800 dpm (10 µl) of [2,4,6,7-3H]-estradiol (Dupont/New England Nuclear, Wilmington, DE, USA) in 10 mM phosphate-buffered saline (PBS; pH 7.4) were added to fecal matter and
the samples were processed as described above. The residue was dissolved in 300 µl of PBS and the radioactivity of aliquots (100 µl) was measured by liquid scintillation spectrometry. The recovery of the extraction procedure was 85.8 ± 1.57% (mean ± SD, n = 5).

The residue was dissolved in 300 µl of PBS. The concentration of estradiol in serially diluted samples was determined by RIA. Aliquots (50 µl) were mixed with 50 µl (about 25,000 dpm) of [2,4,6,7-3H]-estradiol in PBS and 50 µl of a rabbit anti-estradiol-serum in PBS containing 50 mM disodium EDTA. The antiserum was supplied by Prof. K. Wakabayashi (Gunma University, Japan). After incubation overnight at 4°C, 100 µl of dextran-coated charcoal suspension was added. The mixture was incubated for 60 min at 4°C, and then centrifuged at 3,000 × g for 30 min at 4°C. The radioactivity of the resulting supernatant (200 µl of aliquots) was measured by liquid scintillation spectrometry. Estradiol-17β obtained from Sigma (St. Louis, MO, USA) was used as a reference. The assay was validated for fecal extracts by determining the degree of parallelism between the calibration curve for the extracts and the standard curve, as well as by quantitative recovery of estradiol (19.6–157 pg) added to pooled fecal extracts. The intra- and

Fig. 1. Inhibition curves obtained in estradiol radioimmunoassay for estradiol-17β (●) and fecal extracts in females of the migratory Reed Bunting Emberiza schoeniclus (▲) and the non-migratory Meadow Bunting Emberiza cioides (■). Each point and vertical line represents the mean ± SEM for triplicate determinations. Fecal matter was collected during 10:00 hr–15:00 hr from E. schoeniclus females that had been maintained under a 12 hr light/12 hr darkness photoperiod and from E. cioides females that had been maintained under a 14 hr light/10 hr darkness photoperiod. The weights of fecal matter from E. schoeniclus and E. cioides were 1.00 g and 1.03 g, respectively. Fecal estradiol content was determined as described in Materials and methods.
interassay coefficients of variation were 5.5% and 8.8% (both \(n=4\)), respectively. The estradiol levels are expressed in pg/mg of dry feces and not corrected using the data of recovery through extraction procedure.

**Results**

Estradiol immunoreactivity was detected in extracts from the feces of the Reed and Meadow Bunting females using RIA (Fig. 1). The calibration curve for serially diluted samples was almost parallel to the standard curve. Estradiol (19.6–157 pg) added to pooled fecal extracts was quantitatively recovered (Fig. 2). The slope of the regression line was 0.976 and the coefficient of correlation between the amounts added and those assayed was 0.998 \((P<0.001)\). The Y-intercept value, which represented the endogenous estradiol levels in the pooled fecal extracts, was 32.5 pg.

In females of the Reed Bunting maintained under increasing daylength from LD 10:14 to LD 15:9, the fecal estradiol levels were low at LD 10:14 and LD 11:13, and increased significantly \((P<0.01)\) at LD 12:12 (Fig. 3). Subsequently, the levels at LD 14:10 decreased significantly \((P<0.01)\). The fecal estradiol levels in females of the Meadow Bunting exhibited changes similar to those in the Reed Bunting; the levels peaked at LD 12:12 (Fig. 4).

**Discussion**

The present data demonstrate that immunoreactive estradiol is present in the feces of
Fig. 3. Effects of increasing daylength on fecal estradiol content in the migratory female Reed Bunting *Emberiza schoeniclus*. Each point and vertical line represents the mean±SEM (n=3-6/group). The data were expressed as pg/mg of dry feces. The photoperiod conditions were changed stepwise from 10 hr light/14 hr darkness (LD 10:14) to LD 15:9; the portion of the light period was increased at 30 min/week. Fecal estradiol content was determined as described in Materials and methods. Statistical comparison of group means was performed by Duncan's multiple range test or Dunn's test. The fecal estradiol levels at LD 12:12 were significantly (P<0.01) higher than those under other photoperiod conditions.

females of both the Reed Bunting and the Meadow Bunting. The method used in the present study for determination of fecal estradiol levels was validated for the following reasons; (i) the calibration curve for serially diluted fecal extracts was parallel to the standard curve (Fig. 1), and (ii) estradiol added to pooled fecal extracts was recovered quantitatively (Fig. 2).

Previous studies using Japanese Quails have demonstrated that measurement of sex steroid hormones excreted into feces enables estimation of the plasma levels of these hormones (Bishop & Hall 1991). In female Reed Buntings that were maintained under increasing daylength from LD 10:14 to LD 13:11, the levels of estradiol in both plasma and feces increased significantly (Nakamura & Shimada 1988; Fig. 3). Thus, the fecal estradiol levels appear to reflect the levels circulating in plasma.

Previous studies have obtained evidence to suggest that nocturnal migratory restlessness is induced by photostimulation (Farner 1950, Farner et al. 1954, Nakamura & Ito 1982, Nakamura & Kitahara 1983). In Reed Buntings maintained under increasing daylength from LD 10:14 to LD 15:9, active nocturnal restlessness appears between LD 13:11 and LD 14:10, however, in Meadow Buntings, nocturnal restlessness is not observed under these conditions (Nakamura & Ito 1982). The present data indicated that in females of both the migratory Reed Bunting and the non-migratory Meadow Bunting, the fecal estradiol levels increased at LD 12:12 and subsequently decreased (Figs. 3 and
Masanori T. Itoh and Tsukasa Nakamura

Fig. 4. Effects of increasing daylength on fecal estradiol content in females of the non-migratory Meadow Bunting *Emberiza cioides*. Each point and vertical line represents the mean ± SEM (n = 3-5/group). The data were expressed as pg/mg of dry feces. The photoperiod conditions were changed from 10 hr light/14 hr darkness (LD 10:14) to LD 15:9; the portion of the light period was increased at 30 min/week. Fecal estradiol content was determined as described in Materials and methods. Statistical comparison of group means was performed by Duncan's multiple range test or Dunn's test. The fecal estradiol levels at LD 12:12 were significantly (P < 0.01) higher than those under other photoperiod conditions.

Thus, estradiol may not be involved in the control of vernal migration in the migratory female Reed Bunting, as is the case in the White-Crowned Sparrow (Schwabl & Farner 1989). It has been reported that implants of estradiol are ineffective in triggering vernal premigratory fattening in ovariectomized White-Crowned Sparrows (Shwabl & Farner 1989), although ovariectomy before the vernal migration diminishes premigratory fattening (Schwabl & Farner 1989) and estradiol levels in plasma increase during the migratory period (Wingfield & Farner 1978).

It has been suggested that androgen, such as testosterone and 5α-dihydrotestosterone, may be an endocrine regulator of vernal migration in both sexes (Rankin 1991). Implants of testosterone are able to reinstate vernal premigratory fattening in ovariectomized or castrated White-Crowned Sparrows (Stetson & Erickson 1972, Schwabl *et al.* 1988). Plasma levels of androgenic steroids increase prior to and during the spring migratory period in both sexes of the White-Crowned Sparrow (Wingfield & Farner 1978). In the male Reed Bunting maintained under increasing daylength from LD 10:14 to LD 15:9, fecal androgen levels increase at LD 12:12, just prior to the period of active nocturnal restlessness, and relatively high androgen levels are maintained during active restlessness (Nakamura *et al.* 1996). In contrast, no significant change in fecal androgen levels has been observed in males of the non-migratory Meadow Bunting under these conditions (Nakamura *et al.* 1996). Furthermore, in the male White-Crowned Sparrow, it has been
demonstrated that testosterone acts on vernal premigratory fattening by increasing the release of prolactin (Yokoyama 1976, 1977). The significance of androgen in vernal migration of the female Reed Bunting should be investigated further.

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References

Yokoyama, K. 1977. Hypothalamic and hormonal control of photoperiodically induced vernal functions in the
渡り性オオジュリオンと非渡り性ホオジロの排泄物中エストラジオール含有量に対する光周期の影響

性ステロイドホルモンの生殖腺からの分泌は光周期による影響を受けることは数多くの鳥類において知られている (Wolfson 1945, Farner et al., 1983, Wingfield 1983)。また、排泄物中の性ステロイドホルモンを定量することによって、血液中の性ステロイドホルモンレベルを推定することが可能である (Bishop and Hall 1991)。光周期を徐々に変化させた条件下で渡り鳥であるオオジュリオン Emberiza schoeniclus と留鳥であるホオジロ Emberiza cioides を飼育し、雌において性ステロイドホルモンの 1 つであるエストラジオールの排泄物中含量がどのような変化をするのかを調べた。

オオジュリオンとホオジロを山梨県甲府市郊外で捕獲した後、各々を 1 羽ずつケージに入れ、実験室で飼育した。飼育条件は気温 22℃、湿度 50% ではほぼ一定にし、光周期のみを 10 時間明期、14 時間暗期 (LD 10 : 14) から LD 15 : 9 まで、1 週間に 30 分ずつ暗期を短く変化させた。そして飼育期間中に両種の雌、3～6 個体の排泄物を定期的に採取した。これらの排泄物に一定量の蒸留水を加え、ホモジェナイズし、これを遠心した後、上清をジエチルエーテルで抽出し、この抽出物中のエストラジオールをラジオイムノアッセイにより定量した。

その結果、雌のオオジュリオンにおける排泄物中のエストラジオール含量は LD 10 : 14 と LD 11 : 13 では低いレベルであったが、LD 12 : 12 で急激に増加し、高レベルは LD 13 : 11 で観察された。その後、LD 14 : 10 で急激に減少し、LD 15 : 9 でも低いレベルであった。

ほぼ同様の排泄物中エストラジオール含量の変化が雌のホオジロでも観察された。したがって、オオジュリオンとホオジロの両種の雌では、他の鳥類と同様に、卵巢からのエストラジオールの分泌は光周期を短日から長日へ変化することによって上昇することと、長期間の長日処理は生殖腺からのエストラジオールの分泌を低下させることが示唆される。ミヤマントド Zonotrichia leucophrys gambelii を用いた研究結果 (Schwabl & Farner 1989) から示唆されているように、オオジュリオンにおいてもエストラジオールは春の渡りに関与していないと考えられる。