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Original article

Regulation of seasonality in the migratory male blackheaded bunting (*Emberiza melanocephala*)

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Abstract – The present study was carried out on a Palearctic-Indian migratory species, the blackheaded bunting *(Emberiza melanocephala*), to understand the importance of photoperiodism and circannual rhythms in determining seasonality in changes in body mass and testis size in birds. An initial experiment determined the effects of duration and intensity of light on photoperiodic induction. The birds were exposed to different photoperiods (hours of light:hours of darkness; 11.5L:12.5D, 12L:12D, 12.5L:11.5D and 13L:11D) at the same (~ 450 lux) light intensity, and to 13L:11D at different light intensities (50-, 100-, 400-, 800- and 1000-lux). The induction and subsequent regression of photoperiodic responses were dependent upon duration and intensity of the light period until these reached threshold. A second experiment investigated if an endogenous seasonal rhythm underlies photoperiodism in buntings. Birds maintained since February on a 8L:16D photoperiod (a non-inductive short day length invariably used to ensure photosensitivity in photoperiodic species) were subjected periodically to 16L:8D (a long day length), one group every month from mid-March to mid-August. The magnitude of long day response in body mass and testes decreased as the duration of the short days progressed, but testicular response was restored in birds that were exposed to long days in July and August. The birds exposed simultaneously to short, long, and natural day lengths for 32 weeks underwent an induction-regression cycle under long days and natural day lengths, but not under short days in which a decrease in body mass occurred after about 20 weeks. The last experiment examined the importance of latitudinal migration on photoperiodism, by comparing the response to long days of three groups which included birds from populations those were held in the outdoor aviary for 1 or 2 years at 27° N and those immediately arrived from their breeding grounds (~ 40° N). There was no difference in the photoperiodic induction among the three groups, indicating that neither experience to changing photoperiods during a migratory journey, nor to long photoperiods at breeding grounds, were critical for a subsequent response (initiation-termination-reinitiation) cycle. Taken together, these findings suggest that (1) the blackheaded bunting has its own endogenous timing program, which is regulated by the photoperiod, and (2) the photoperiodic programs of bunting are flexible enough to accommodate variations in the amplitude of environmental cycles. Thus, it appears that photoperiodism has evolved independently of the evolution of migration in this species.

bunting / intensity / photoperiod / photoperiodic response system / photosensitivity / seasonality

1. INTRODUCTION

Day length away from the equator changes with the season, and light intensity at any one time of day changes with the weather. Since the pioneering studies of Rowan [1] on Slate-colored Juncos *(Junco hyemalis),* the role of day length (or photoperiod) as a

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major source of temporal information regulating seasonal responses has been demonstrated in many bird species (see especially, [2–8]). Relatively less is known of photoperiodism in tropical birds (but see [9, 10]), and especially of latitudinal migrants that overwinter at lower latitudes. However, we studied photoperiodism in two Palearctic-Indian migrants, the blackheaded bunting *(Emberiza melanocephala)* and the redheaded bunting *(Emberiza bruniceps)*, that breed in summer in west Asia and east Europe (~ 40° N) and over-winter in India (25° N). They are highly photosensitive; photoperiods ≥ 12 h induce body fattening and gonadal growth and development, and subsequently spontaneous regression and photorefractoriness follow if the birds are exposed to stimulatory photoperiods for ca. 12 weeks or more [11–14].

In the nature, birds experience gradual changes in the duration and intensity of daily light, and so they should be able to "read" precisely such gradual changes. How dynamic the photoperiodic response system is can be studied experimentally by subjecting individuals to a range of photoperiods and light intensities. In one such study on Japanese quail *(Coturnix c. japonica)*, Follett and Maung [15] found a relationship between the length of photoperiod and rate of photoperiodic induction. The rate of testicular growth was slower by 50% in birds exposed to 12L:12D (12 h of light:12 h of darkness) compared to 14L:10D, 16L:8D and 20L:4D in which full growth had occurred; a near full growth occurred in 13L:11D. Similarly, from their experiments on European starlings *(Sturnus vulgaris)* Bentley et al. [16] concluded a relationship between the light intensity and rate of photoperiodic induction. Starlings exposed to 18L:6D at 3-, 13-, 45- and 108-lux light intensities responded as if they were exposed to 11L:13D, 13L:11D, 16L:8D, and 18L:6D, respectively. Hence, the first goal of this study was to determine the induction-regression response of blackheaded buntings *(Emberiza melanocephala)* to a range of photoperiods and light intensities. The blackheaded bunting differs from the widely studied Japanese quail in being a strong latitudinal migrant (quails are semidomestic), and in having a strong self-sustained photoperiodic clock system [17]; in contrast, quails have a weak self-sustained clock system [18].

Apart from photoperiodism, which is mediated by a circadian rhythm of photoperiodic photosensitivity (CRPP) [3, 7, 17, 19], the other mechanism involved in the regulation of avian seasonality is the circannual rhythm generation [20], as evidenced by studies on spotted munia *(Lonchura punctulata)* [21] and stonechats *(Saxicola torquata)* [22]. A circannual rhythm, which is characterized by a spontaneous initiation-termination-reinitiation of a response, is usually not shown in several photoperiodic species, such as the white-crowned sparrows *(Zonotrichia leucophrys gambelii)* and the blackheaded bunting *(Emberiza melanocephala),* since they exhibit absolute photorefractoriness following long day photostimulation [23, 24]. Our second goal was, therefore, to examine if an endogenous long-term rhythmicity still underlies photoperiodism in the blackheaded bunting by comparing the magnitude of photoperiodic induction in a batch of buntings that were maintained on non-stimulatory short days (8L:16D) and subjected periodically to long days (16L:8D). If demonstrated to be true, this will present a unifying view of photoperiodic and circannual rhythm regulation of long-term seasonal functions in vertebrates.

An issue often inadequately addressed is the plasticity of the photoperiodic response system. In other words, how tightly is the photoperiodic response system coupled to seasonal variations in day length that a species experiences in its natural habitat? A migrant species is a useful model to determine plasticity of the photoperiodic response system, since it experiences varying photoperiodic conditions each year – one at its breeding ground, other at its wintering ground, and still other (consistently varying day lengths) during migration between the

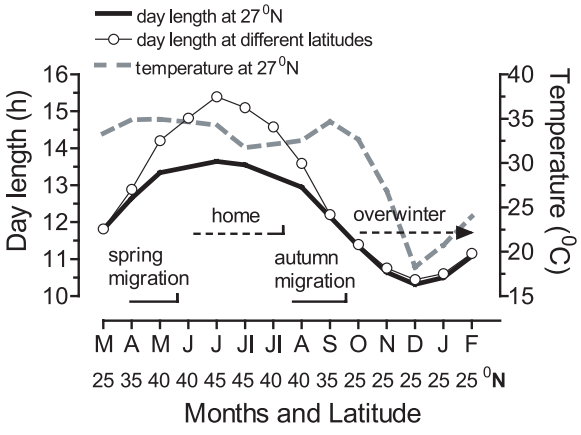

Figure 1. Variations in day length (sunrise to sunset on 16th of the month) and in mean temperature at 1200 h during different months of a year. Mean temperature, shown for one-year during the period of this study, was calculated for 15 days from 8th to 22nd of every month. Also plotted (circles) is the variation in day length (sunrise to sunset 16th of the month) at different latitudes that blackheaded bunting likely experiences during the year in its migration, and at breeding (~ 40–45° N) and wintering (25° N) grounds, based on Ali and Ripley [32]. Buntings leave breeding grounds in late July/August, and start returning to their breeding grounds in early April (spring migration). Birds at breeding grounds and wintering grounds (25° N) are noticed beginning from May and October, respectively.

wintering and breeding grounds. For example, the blackheaded bunting flies south in the autumn (fall or autumnal migration) to their wintering grounds at ~ 25° N, and then returns north in the spring (spring or vernal migration) to their breeding grounds at ~ 40° N. Therefore, we compared the photoperiodic response of buntings held captive at 27° N for 1–2 years, and so were deprived of experiencing photoperiodic cycles that would have been experienced by migrating birds, with that of new arrivals that had experienced usual photoperiodic variations.

2. MATERIALS AND METHODS

The experiments were performed on male migratory blackheaded buntings (*Emberiza melanocephala*) obtained in February from overwintering flocks at 25° N. Unless otherwise indicated, the birds were held under short days (8L:16D), until exposed to an experimental lighting condition, to ensure their photosensitivity to light. An 8L:16D light regime was considered as short day since day lengths which buntings experience at their breeding ground scattered from ~ 40–45° N can be as short as ~ 8.5 h per day (Fig. 1). Also, our previous studies have shown the absence of photostimulation and maintenance of photosensitivity under 8L:16D [11, 12]. Such short day pretreated birds were referred to as photosensitive birds.

2.1. Experiment 1. Effects of increasing duration and intensity of light periods

This experiment, beginning 12 June 2000, addressed the first goal of the study, and contained two sets of experiments performed on photosensitive birds. The first set examined responses (body fattening and subsequent gain in body mass, and testis recrudescence) to photoperiods that were close to the threshold photoperiod for photoperiodic induction in buntings [12]. Groups of birds (n = 6–8 per group) were exposed

for 12 weeks to 11.5L:12.5D, 12L:12D, 12.5L:11.5D and 13L:11D photoperiods with a light intensity of ~ 450 lux at the perch level. In the second set, which examined the effects of a varying intensity on photostimulation, the birds (n = 5–6 per group) were exposed for 11 weeks to 13L:11D, a photoperiod that induces a full response in buntings [25], at 50-, 100-, 400-, 800- and 1000-lux light intensities.

2.2. Experiment 2. Seasonal changes in photoperiodism

This experiment asked the question as to whether the inductiveness of the photoperiodic response system is altered seasonally. We attempted to answer this by examining whether or not birds on short days, which would have not undergone the induction-regression-refractoriness response cycle, would exhibit a full response when they were periodically subjected to a long day. Beginning from mid-March 2000, and continuing every month for the next six months (mid-March to mid-August), a group (n = 5–11) of buntings from the stock maintained on 8L:16D since mid February was transferred to 16L:8D for a period of 10 weeks. Simultaneously, from mid-March, we subjected birds (n = 7–14) to 8L:16D, 16L:8D and NDL (natural day length) for 32 weeks, covering the entire duration of the long day transfer experiment.

2.3. Experiment 3. Plasticity of the photoperiodic response system

This experiment investigated whether buntings deprived from experiencing day lengths that they would experience during migration and at the breeding grounds (Fig. 1) would show reinitiation of body fattening and testicular growth in the subsequent year as if they were similar to those immediately returned from the breeding grounds. A batch of buntings were held captive in our out door aviary at 27° N since February 2000 and 2001. In March 2002, a group of birds from these two captive stocks [years 2000 (n = 8) and 2001 (n = 9)] along with a group (n = 8) of birds from newly arrived stock (caught in February 2002 and acclimated to aviary conditions since then) were exposed to 16L:8D for 3 weeks.

During the photoperiodic treatment, the birds were held in groups of 3 or 4 individuals per cage (size – 45 × 25 × 25 cm) in light-proof boxes (size – 138 × 60 × 56 cm) providing white light by fluorescent (Philips) tubes. Automatic time switches controlled the periods of light and darkness. Light intensity was always measured at the perch level by an Eurisem Technics EP 628 digital Lux meter. Temperature was not strictly regulated, but our indoor photoperiodic chambers are well aerated and the temperature inside these chambers does not vary more than 1–2 °C from the room temperature. However, we recorded temperature of the outdoor aviary daily three-times a day (0800, 1200 and 1700 h) during the course of the experiment. Figure 1 shows variations in mean temperature at 1200 h (calculated for 15 days from 8th to 22nd of every month) and day length (on the 16th of every month) at 27° N covering the experimental period, and also in day length what buntings might experience in the wild when migrating and at their breeding and wintering grounds. The induction and subsequent regression of a photoperiodic response were measured by recording changes in the body mass and testis size at the beginning and the end of the experiment, and also at appropriate intervals during the experiments (see figures). Body mass was measured using a top pan balance to an accuracy of 0.1 g. The testicular response was assessed by laparotomy under local anesthesia (for details, see [26]). The dimensions of the left testis were recorded, and testis volume was calculated from $4/3\pi ab^2$, where a and b denote half of the long and short axes, respectively. The data are presented as means and SEs. They were analyzed statistically using 1-way and 2-way analysis of variance (ANOVA) with or without repeated measures, as appropriate, followed by the post hoc Newman-Keuls

test, if ANOVA indicated a significance of the difference. Two groups at one time-point were compared using the Student *t*-test. Significance was taken at $P < 0.05$.

3. RESULTS

3.1. Experiment 1. Effects of increasing duration and intensity of light periods

3.1.1. Effects of increasing duration

Figures 2a and 2b show significant gain and loss in body mass, and growth and regression of testes under all four photoperiods ($P < 0.0001$, 1-way RM ANOVA). A comparison by 2-way ANOVA of data on body mass from all experiments indicates a significant effect of both the photoperiod ($F_{(3,140)} = 3.85$, $P < 0.01$) and the duration of exposure ($F_{(6,140)} = 17.63$, $P < 0.0001$). At the end of 2 weeks, there was a significant gain ($P < 0.05$, Newman-Keuls post hoc test) in the body mass of the birds exposed to 13L:11D (Fig. 2a). In the other three groups, a similar gain in body mass ($P < 0.001$, Newman-Keuls post hoc test) was found at the end of 4 weeks, when the birds in the 13L:11D had a near-maximum gain (Fig. 2a). At the end of 6 weeks, all birds had maximum (or near maximum as in 11.5L birds) gain in body mass, which they maintained until 8 weeks of exposure. Thereafter, there was a spontaneous decrease in fat stores and subsequent loss in body mass, and at the end of 12 weeks the mean body mass of all but the 13L:11D groups was not different from their initial means.

A similar pattern was observed in the testicular response. There was a significant ($P < 0.001$, 1-way RM ANOVA) growth-regression of the testes in all photoperiods. However, the rate of testis recrudescence was duration-dependent: 11.5L < 12L < 12.5L < 13L (Fig. 2b). At the end of 4 weeks, testis growth was 80-, 33-, 25-, and 10-% of the maximum growth attained in this experiment under 13L:11D, 12.5L:11.5D, 12L:12D and 11.5L:12.5D, respectively. Also, the birds exposed to 11.5L and 12L had more variable response. The peak testicular response in respective photoperiods was maximal at the end of 8 weeks, although mean testis size of 11.5- and 12L birds were still 50% and 75%, respectively, compared to that of 12.5L or 13L birds at this time (Fig. 2b).

3.1.2. Effects of increasing intensity

Figures 2c and 2d show differential effects of light intensity on photoperiodic induction. A complete photoperiodic response cycle, represented by significant ($P < 0.0001$, 1-way RM ANOVA) gain-loss in body mass and growth-regression in testes, occurred in all groups except the one exposed to 50 lux. At 50 lux light intensity, a significant ($P < 0.05$, 1-way RM ANOVA) gain in body mass occurred after 11 weeks of exposure, resulting in a 7-week delay in photoperiodic induction in this group as compared to the other groups in the experiment (Fig. 2c). However, because of the large individual variations, there was no statistical significance of difference, when this group was compared with other groups at higher light intensities (100-, 400-, 800- and 1000-lux; Fig. 2c). At 100-lux light intensity, all birds attained full response, and were not different from those in the other three groups at higher light intensities (Fig. 2c). However, there were some differences in the response pattern between these four groups, such as: (i) a light intensity of 1000 lux appeared to accelerate the photoperiodic induction, and the peak in response was advanced by at least 2 weeks; (ii) the peak response in the 1000-lux group was slightly smaller; and (iii) the gain-loss response curve for body mass of the 400-lux group was slightly different (Fig. 2c).

A similar testicular response to various light intensity treatment was also found (Fig. 2d). A significant increase ($P < 0.05$, 1-way RM ANOVA) in testis size was found at the end of 2 weeks in all the groups, except the one exposed to 50 lux (Fig. 2d).

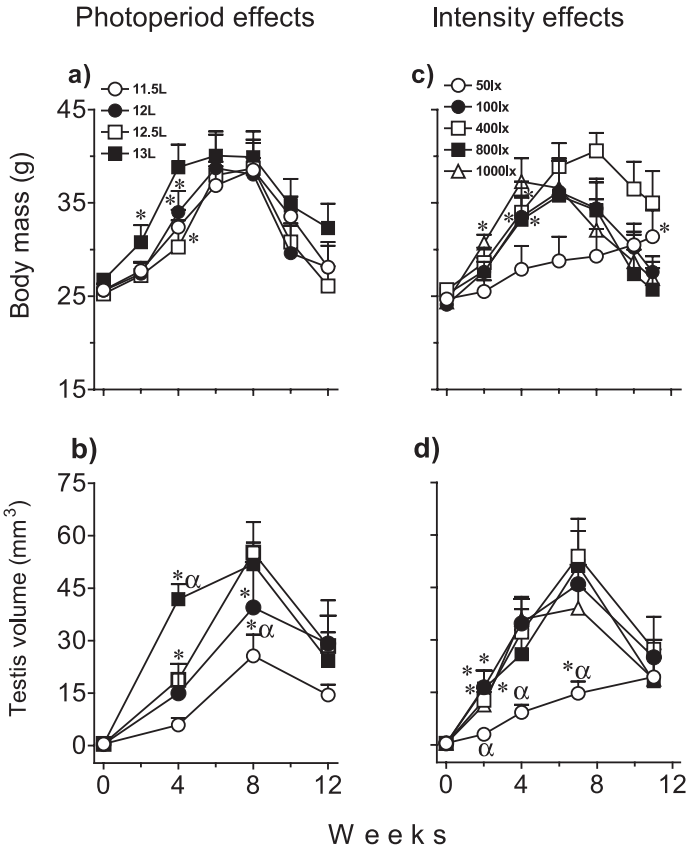

Figure 2. Mean (± s.e.m) body mass and testis volume of the blackheaded bunting in response to different photoperiodic conditions. Left (a, b) – photoperiod effects: photosensitive birds (n = 6–8) were exposed to different photoperiods (ranging from 11.5-, 12-, 12.5- and 13-h light per day) for 12 weeks. Note: (i) a faster photoinduction in 13L:11D, significantly different from other groups in testis response, (ii) a difference in the response pattern between body mass and testes under 11.5L:12.5D, and (iii) a similarity in the induction-regression response curve. Right – intensity effects (c, d): photosensitive birds (n = 5–6) were exposed to 13L:11D for 11 weeks at 50-, 100-, 400-, 800- and 1000 lux light intensities. There was an intensity-dependent photoinduction, and similarity in the induction-regression response when the threshold was reached. Significance of difference, $P < 0.05$; * different from initial observation; α, different from two or more groups.

Birds exposed to 50 lux showed a significant increase in testis size by the end of 8 weeks, but did not attain full testicular response (Fig. 2d). Hence, the testicular response to the 50-lux light intensity was different from the testicular response to higher light intensities. A 2-way ANOVA reveals a significance in effects of both the light intensity (body mass: $F_{(4,147)} = 5.92$, $P = 0.002$; testes: $F_{(4,105)} = 7.57$, $P = 0.0001$) and the duration of exposure (body mass: $F_{(6,147)} = 11.99$, $P < 0.0001$; testes: $F_{(4,105)} = 38.24$, $P < 0.0001$).

3.2. Experiment 2. Seasonal changes in photoperiodism

The results are shown in Figure 3. Buntings exposed to long days underwent a complete induction-regression cycle (Figs. 3a

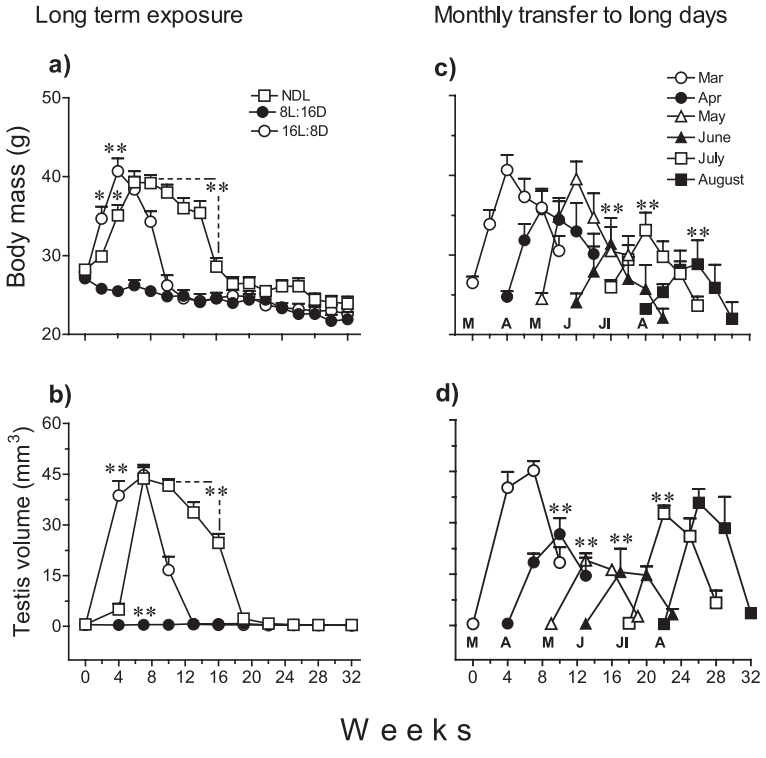

Figure 3. Changes (mean ± s.e.m) in body mass and testis volume of the blackheaded bunting held under different photoperiodic conditions. Left (a, b) – long-term exposure: photosensitive bunting were held under natural day length ($n = 7$) at 27° N or exposed to short- (8L:16D; $n = 13$) and long days (16L:8D; $n = 14$) for 32 weeks, beginning mid-March. There was a complete induction-regression cycle under natural day lengths and long days but no photoinduction under short days (however, note a significant decrease in body mass, week 24 onwards). Right (c, d) – monthly transfer to 16L:8D: photoinduction-regression of body mass and testes in photosensitive bunting that were maintained under 8L:16D since February, and each month beginning from mid-March, a group ($n = 5$–11 per group) of birds were subjected to 16L:8D for 10 weeks. Note a difference in the response during different months; first a consistent decrease in the magnitude of response of both body mass and testicular responses, and then restoration of testicular response in July and August. The asterisks indicate a significance of difference at $P < 0.05$. * Compared with an initial observation; ** compared with other groups.

and 3b). That is, they first fattened and gained in body mass and the testes recrudesced, and then underwent spontaneous depletion in fat stores and loss in body mass, and testicular regression (Figs. 3a and 3b). A similar induction-regression cycle in body mass and testes was induced in response to consistently lengthening natural day lengths from March to May, although there was a delayed response under natural day length due to gradual increments in daily light hours. The peak response was almost identical in both the artificial long days and natural long days. In contrast, the birds exposed to short days did not respond, and they maintained unstimulated body mass and small testes (Fig. 3a). However, by week 24 of short day exposure, there was a significant decrease in body mass ($P < 0.05$, Newman-Keuls post hoc test).

An induction-regression response curve, as is typical of response to long days (Figs. 3a and 3b), was stimulated in buntings that were transferred from short days to long days in the middle of every month from mid-March to mid-August (Figs. 3c and 3d). The induction-regression response curves of all groups were significant ($P < 0.05$, 1-way RM ANOVA), but the peaks in response were significantly different across the groups (body mass: $P < 0.01$, 1-way RM ANOVA; testes: $P < 0.0001$, 1-way RM ANOVA). The peak gain in body mass was similar in March, April and May birds, but was lower in June, July and August birds (Fig. 3c); the peak body mass gain in August birds was significantly different ($P < 0.05$, Newman-Keuls post test) from that of March or May birds. Also, initial average body mass of birds taken from short days progressively decreased from March to August, but these were not statistically significant ($P = 0.0784$, 1-way ANOVA). Although there was no difference in the initial size of the testes among different groups, the peak testicular growth became progressively (significantly) smaller ($P < 0.0001$, 1-way ANOVA) in April, May and June birds, but then was restored in July and August birds (Fig. 3d). The peak testis growth of the March group was significantly different ($P < 0.05$, Newman-Keuls post hoc test) from all, but the August groups; the June group had the smallest peak (Fig. 3d).

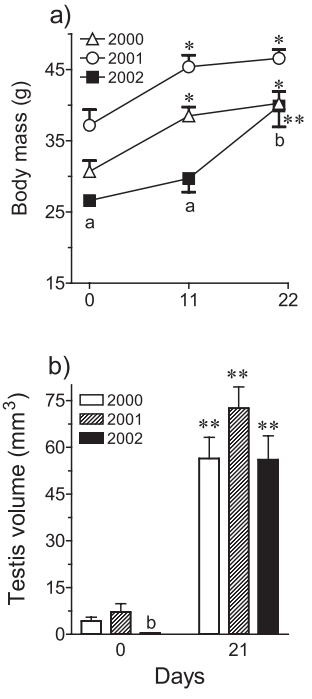

Figure 4. A comparison of photoinduction in body mass and testes in three groups of black-headed bunting that were held either captives in an outdoor aviary (under NDL) at 27° N for 1 year (since February 2001) or 2 years (since February 2000), or caught from a freshly arrived batch of birds (February 2002). Each point (a) or bar (b) represents a mean of a group of birds (n = 8–9), and vertical line on it indicates the standard error. Note a small group difference in body mass and testis size at the beginning of the experiment. Asterisks or subscribed alphabets indicate a significance of difference at $P < 0.05$. * Compared to an initial observation; ** compared to a preceding observation; (a) among all three groups; (b) one group is different from the other two groups.

3.3. Experiment 3. Plasticity of the photoperiodic response system

Buntings showed a significant induction ($P < 0.0001$, 1-way RM ANOVA) and attained a full photoperiodic response under long days, regardless of whether they came from the stocks that were held captive for 1 or 2 years at 27° N or from the freshly arrived stock (Fig. 4). There were, however, a few noticeable differences. First, at the beginning of the experiment, the body mass and testis size of the three groups were different (body mass: $F_{(2,22)} = 10.99$, $P < 0.001$; testes: $F_{(2,22)} = 3.63$, $P = 0.043$; 1-way ANOVA). Post hoc comparison of the groups reveals that freshly arrived birds had significantly ($P < 0.05$, Newman-Keuls post hoc test) lower body mass and smaller testes than the group held captive for 1 year. There was also a significant difference ($P < 0.05$, Newman-Keuls post hoc test) between 1- and

2-year captives in body mass, but not in testis size (Figs. 4a and 4b). Compared to the newly arrived birds, the body mass was higher and the testes were larger in the group held captive for 2 years, but the means were not significantly different. Second, the rate of photoperiodic induction of body mass gain was different between captives and freshly arrived birds during the first and second halves of the experiment. In the first half of the experiment (day 0 to day 11), the gain in body mass was 23.70 ± 4.36% ($n = 9$) and 26.50 ± 4.31% ($n = 8$) in 1- and 2-year captive birds, respectively, compared to 11.20 ± 5.28% ($n = 8$) in newly arrived birds. But, in the second half of the experiment (day 12–day 22), captives further gained only a little body mass (~ 4 to 7%), but new birds gained much greater body mass (~ 38%), compensating for the smaller gain in the first half of the experiment. At the end of the experiment, gain in body mass among the different groups was as follows: 33.10 ± 7.91% (2 year captives, $n = 8$), 27.70 ± 5.74% (1 year captives, $n = 9$) and 49.80 ± 10.16% (fresh birds, $n = 8$). Thus, all the three groups had fully responded, but because of higher initial values 1-year captives appeared to have greater body mass and larger testes (Fig. 4).

4. DISCUSSION

Seasonality in reproduction, represented by the initiation-termination-reinitiation of physiological processes, is regulated by two mechanisms. One is photoperiodism, in which environmental photoperiod times the component events of seasonality. The other is circannual rhythm generation, in which a self-sustained endogenous rhythmicity of ca. 1-year times these component events. These two mechanisms may not be mutually exclusive and, in fact, might interact closely, albeit in a species-specific manner. However, several studies support an alternative proposition, that photoperiodism and circannual rhythm generation evolved as separate mechanisms. For example, (1) post-reproductive photorefractoriness is maintained, and so a second testicular cycle is not initiated, in the white crowned sparrows as long as they are kept on long days [26], and (2) circannual rhythm is expressed in low-latitude and equatorial species that are considered usually as non-photoperiodic [20, 21]. Our current findings should be viewed against this background. First, we established that seasonality in the blackheaded bunting is regulated by a dynamic photoperiodic response system (experiment 1; Fig. 2). Second, our data indicate the probability of an endogenous seasonal rhythm underlying the photoperiodism in this species (experiment 2; Fig. 3). Third, we provide evidence (experiment 3; Fig. 4) that the photoperiodic program of the bunting is flexible enough to produce a complete response (initiation-termination-reinitiation) cycle at a relatively lower amplitude day-night cycle of 27° N (Fig. 1).

The results from experiment 1 are consistent with previous findings on this [12] and other species ([13, 15]; see reviews [7, 23]), and suggest that the blackheaded bunting possesses a highly dynamic photoperiodic response system, which can selectively respond to externally imposed environmental light conditions. The following observations are of interest. (1) Regulation of body mass and testes appear to be two separate photoperiodic phenomena with different thresholds, as argued earlier [27]. An 11.5L photoperiod produces maximal, albeit at the slower rate, body mass gain, but only half-maximal testis growth (Figs. 2a and 2b). Such a response-specific photoperiodism could be adaptive, since in nature the fattening and gain in body mass, which are critical to spring migration, precedes the time of testis recrudescence [28]. (2) Light intensity influences the threshold photoperiod; a 13L photoperiod at 50 lux induces only half-maximal photoperiodic induction and does so more slowly (Figs. 2c and 2d). This is consistent with the results of Bentley et al. [16] on European starlings which show that lower intensities proportionally decrease the perception of the length of day.

(3) The relationship between the strength of photoperiodic stimulus and photoinduction is not linear. For example, exposure to 1000 lux did not result in greater photoinduction. The mean peak testis size in buntings exposed to 1000 lux was in fact slightly smaller than that from the other three groups (Figs. 2c and 2d), which may indicate that a 1000-lux light intensity in our photoperiodic chambers was somewhat stressful. This might appear surprising when diurnal buntings may be exposed to many thousand of lux during a normal sunny day. However, in nature birds are never exposed continuously to a 1000-lux light intensity. For most of the day, they usually inhabit shade areas (e.g. forest canopies) where they receive natural light at intensities reduced many folds on a log-scale. Interestingly though, this "unnaturalness" of reduced light intensity underneath shade corresponds to that provided in the laboratory by cool white fluorescent lamps [29]. (4) The amplitude of the photoperiodic cycle determines the magnitude of response, but does not change the shape of the induction-regression response curve. Regardless of the length of the light period, the timing of the peak (the highest point in the curve supported by another observation on either side) was similar in all the treatments (Fig. 2). The induction-regression response curve for body mass of birds under 400 lux was slightly different. Possibly, a 13L:11D photoperiod at 400 lux provided the best-suited lighting conditions in our experimental facility.

Experiment 2 provides an interesting insight into the responsivity of the avian photoperiodic system. Typically, a long day species undergoes post-induction spontaneous regression (Figs. 3a and 3b). If the photoperiodic induction must precede spontaneous regression and loss of photoresponsivity (photorefractoriness), then birds exposed to non-inductive short days will never show a decrease or loss in the photoresponsivity (Figs. 3a and 3b). This proposition completely discounts the existence of an endogenous seasonal rhythm of photosensitivity. However, supposing that there is such an endogenous seasonal rhythm, the photoperiodic induction in birds that were maintained on non-inductive short days should vary, depending on the time of the year when they were exposed to long days. In experiment 2, our lighting protocol enabled us to test the probability of an endogenous rhythmicity underlying photoperiodism in the blackheaded bunting. Figures 3c and 3d show a progressive decline in the magnitude of photoperiodic induction in both the body mass and the testes, and a restoration of the peak testicular response in July and August. Restoration of the testicular response indicates the regaining of full photosensitivity, and this is in conformity with the evidence that short days recover photosensitivity in long day refractory birds [5, 23]. A difference in the trend of response between body mass and testes reinforces the argument of difference in the photoperiodic strategies between these two physiological functions.

Since the lower responses in experiment 2 coincide with the months of higher temperature, it is logical to argue that higher temperatures result in higher concentrations of prolactin [30], and high prolactin levels inhibit testis recrudescence since prolactin is antigonadal in the blackheaded bunting [31]. We cannot discount completely the influence of such an endocrine negative feedback driven by prolactin on photoperiodic induction in the absence of a strict temperature control in these experiments. But, we are reluctant to accept such a proposition in view of the fact that temperature variation during the entire experimental period was only about 3 °C, the reduction in the magnitude of testis response was not necessarily limited to months with higher range of temperatures, and the testicular response was restored in July and August when temperature was still high (Fig. 1). In view of these results, however, the magnitude of photoperiodic induction under the lighting conditions of experiment 1 could be assumed to have been greater, since it began on short-day pretreated birds in June when responsivity appears to be

slightly compromised. However, the conclusions drawn from experiment 1, which are based on comparisons between groups having identical photoperiodic history, will remain unchanged.

A similar photoperiodic induction among the three groups of buntings from experiment 3 (Fig. 4) suggests that annual variations in day length at 27° N, which is lower in amplitude by about 2 h than that of the breeding grounds (Fig. 1), were sufficient to regulate seasonality (initiation-termination-reinitiation) in body mass and testes of blackheaded buntings. Such plasticity of the photoperiodic response system appears adaptive since buntings migrate along different latitudes twice a year, and stay for 3–6 months at two different latitudes [32] (Fig. 1). However, we noticed a difference in the body mass and testis size among three groups when we exposed them to long days; the body mass was higher and testes were larger in captives than in newly arrived birds. We do not know precisely the reason for this difference, but can offer some plausible explanations. One explanation is that birds held captive at 27° N advanced their photosensitivity cycle, resulting in initiation of photoperiodic events in response to increasing photoperiods earlier than the newly arrived ones. This is supported by the observation that under 16L:8D, most of the gain in body mass in captives occurred in the first half of the experiment, and most of the gain in body mass in newly arrived birds occurred in the second half of the experiment (Fig. 4a). Clearly, the mechanisms underlying photoinduction were set in motion in captives at the time of their exposure to 16L:8D. Another explanation is that the energy spent during migration by newly arrived birds delayed the photoinduction. We know food-deprivation, which tends to lower energy supply, compromises photoperiodic induction in the blackheaded bunting [26]. Finally, the difference in response was because newly arrived birds were probably the first-year adults. It is known that age does have an effect on testicular size and the testicular cycle [33].

In summary, our findings suggest that the blackheaded bunting has its own endogenous timing program, which is regulated by the photoperiod. The photoperiodic programs of buntings are flexible enough to accommodate variations in the amplitude of environmental cycles. The mechanisms of photoperiodism and circannual rhythm generation appear to be mutually inclusive, and photoperiodism is dominant in species like blackheaded bunting which experiences distinct photoperiodic cycles in nature. Furthermore, photoperiodism appears to be evolved independently of the evolution of migration in this species.

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