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# Life at a different pace: Annual itineraries are conserved in seasonal songbirds

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The duration of life history state (LHS) reflects the adaptive strategy a species has evolved to cope with a changing environment. Inhabitants at different latitudes may thus have significant differences in the rates of metabolic and physiological processes underlying LHSs. Birds, in order to maximize their fitness in the environment in which they live, seasonally switch from one LHS to another during the year. The present study investigated whether an annual itinerary of a species would determine its rate of reaction to inductive long days. We compared the photoinduced cycles of changes in body mass and testes, as indices of migratory and reproductive LHSs, between two long day breeding species, the migratory redheaded bunting and non-migratory Indian weaverbird. Changes in body mass and testis size were measured in photosensitive buntings and weaverbirds ( $n = 7$  each) on short days (LD 8:16) subjected first to 0.5 h weekly light increments until the light period was 13 h per day, and then maintained on LD 13:11 for another 32 weeks. A similar observation was recorded on a group of buntings ( $n = 14$ ) and weaverbirds ( $n = 9$ ) maintained on increasing natural day lengths (NDL; Lucknow, 26°55' N, 80°59' E) for 47 weeks. As predicted, the rates of induction of seasonal cycles under an identical inductive photoperiod were significantly faster in temperate buntings with five annual LHSs than in the subtropical weaverbirds with three annual LHSs. This suggests that annual itineraries of songbirds with which they may have evolved with at their breeding latitudes, determine their response to the external photoperiodic environment.

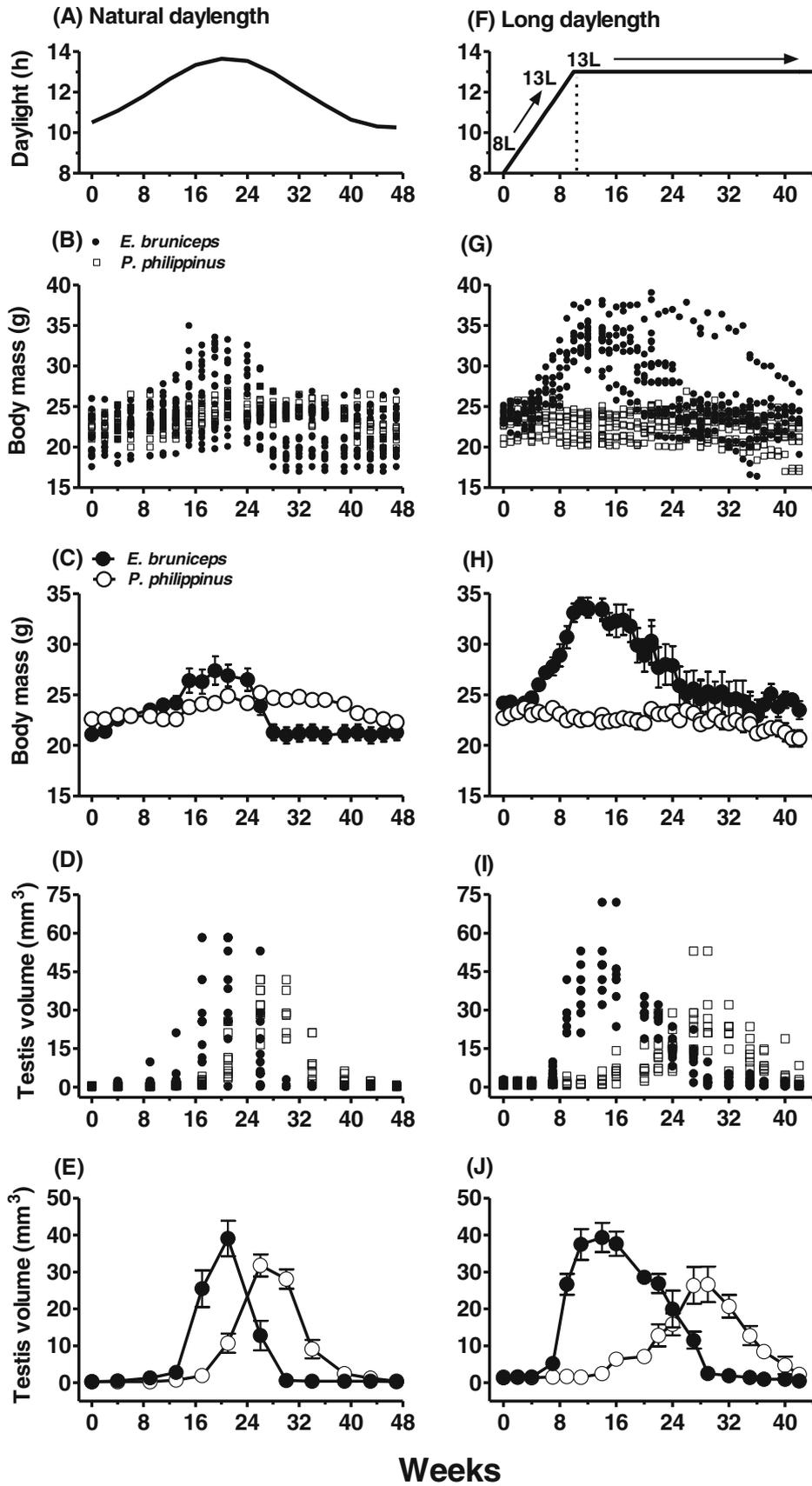
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## 1. Introduction

Most animal species exhibit seasonal activity schedules such that there is no mismatch in timing of their occurrence and the external environment (Helm *et al.* 2009). Seasonal activity schedule is usually identified by a distinct phenotype; this is described as a life history state (LHS) (Wingfield 2008). Each LHS begins and ends on time so that it occupies a specified period in the year, as determined by the interaction of endogenous clocks with the surrounding environment (Gwinner 1996; Wingfield 2008; Budki *et al.* 2012). Hence, the timing and duration of

LHSs during the year (=annual itinerary) can differ both at individual (intraspecific) and species (interspecific) levels within and between habitats (Helm *et al.* 2009). Such flexibility in the annual itinerary of a species ensures synchrony between seasonal life history events (e.g. reproduction, migration) and variable environmental conditions. For example, high-latitude species have relatively rigid seasonal schedules and low-latitude species have flexible schedules (Bradshaw and Holzapfel 2007). Latitudinal differences in breeding seasons can be driven by the photoperiodic environment at those latitudes (Dawson 2007), although differences may exist between populations

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of a species even at the same latitude perhaps due to other ecological factors (Perfito *et al.* 2004, 2005; Caro *et al.* 2005). Thus, a shorter duration and faster rates of gonadal maturation and feather molt (Ricklefs 1980) in a species at temperate latitudes may have evolved in response to a shorter and more intense duration of adequate food supplies, as dictated largely by the photoperiod, temperature and other climatic conditions (Martin 1996). Differences linked with prevailing photoperiod and other ecological factors at respective latitudes can also be seen in the rates of embryonic development of temperate and tropical birds (Cooper *et al.* 2011).

Most, if not all, avian annual itineraries include breeding, molt and non-breeding LHSs. A migratory species has at least two additional migratory LHSs each year, the spring and autumn migrations, placed before and after the reproductive LHS, respectively. Thus, these independently programmed LHSs of an annual itinerary in a species are interlinked, i.e. with a definite timing and duration they follow one another. For example, seasonal cycles in body mass, molt, body plumage (in some species) and migration (in migrants) appear to be closely linked with gonadal phases, so that each of them lasts for a defined duration in relation to the breeding season. A change in the timing and/or duration of one LHS may affect the subsequent LHSs of a species (Wingfield 2008).

The present study compared photoinduced cycles of changes in body mass and testes, as the indices of migratory and reproductive LHSs, between temperate, migratory red-headed bunting (*Emberiza bruniceps*) and subtropical, non-migratory Indian weaverbird (*Ploceus philippinus*). Buntings arrive in India (~25°N) in September/October, overwinter, and return to their breeding grounds in west Asia and East Europe (~40°N) in March/April (Ali and Ripley 1974). Thus, buntings during their overwintering period lasting about half-a-year (October to March) share habitat with weaverbirds, although they widely differ in their breeding latitudes and the number of their annual LHSs. Buntings' annual itinerary is comprised of five LHSs (winter, spring migration, breeding, molt and autumn migration), while weaverbirds' annual itinerary has only three LHSs (non-breeding, breeding and molt). But, both birds are summer (long day) breeders, and use photoperiodic cues in regulation of their seasonal cycles in migration (bunting only) and reproduction (Rani *et al.* 2005, 2007). Here, we asked the question whether annual itineraries with which these two songbird species may have evolved with at their respective

breeding latitudes, would determine their rates of reaction to stimulatory long days and, in turn, the timing of LHSs in the year. If yes, testicular maturation–regression cycle under an identical inductive photoperiod would occur significantly earlier in buntings than in the weaverbirds. Alternatively, the two species would exhibit overlapping reproductive LHS.

## 2. Materials and methods

Groups of adult male redheaded buntings and weaverbirds were maintained on short days (8 h light: 16 h darkness, LD 8:16; L = 400 lux) after their capture in January for the experiment at the Department of Zoology, University of Lucknow, India (27°N). Under short days, they are photosensitive and unstimulated, and maintain normal body mass and reproductively inactive testes (Rani *et al.* 2005, 2007). Birds were maintained in photosensitive unstimulated LHS under short days through the spring migratory (only for bunting) and reproductive LHSs till the end of August. Beginning on 01 September, a group ( $n = 7$  each) of each species was exposed to 0.5 h weekly increments in daily light period (0.25 h each in morning and evening), until the photophase lengthened to 13 h per day (LD 13:11). Thereafter, they were maintained for another 32 weeks on LD 13:11, which is a stimulatory photoperiod for both the species (Rani *et al.* 2005, 2007). Temperature was kept constant at  $22 \pm 2^\circ\text{C}$  (Singh *et al.* 2012). The observations on body mass and testis size, recorded at the intervals of 2 and 4 weeks, respectively, were considered reflective of the migratory (only for bunting) and reproductive phenotypes, respectively (Rani *et al.* 2005, 2007). We measured body mass of each individual using a top pan balance (Kerro, India) to an accuracy of 0.1 g. The size of the left testis was measured by laparotomy under local anesthesia (for details, see Kumar *et al.* 2001). Briefly, left testis was located in the abdominal cavity through a small incision in between the last two ribs on left flank, and its dimensions were measured using a caliper with reference to an accurate scale plotted on a graph sheet. Testis volume (TV) was calculated from  $4/3\pi ab^2$ , where  $a$  and  $b$  denote half of the long and short axes, respectively. Similar observations were recorded for 47 weeks on a group each of buntings ( $n = 14$ ) and weaverbirds ( $n = 9$ ) kept on natural light and temperature conditions (NDL) beginning from January, when they were captured. Thus, they experienced increasing day lengths at Lucknow, India (26°55' N, 80°59' E). Food and water were given *ad*

◀ **Figure 1.** Body mass (B,C,G,H) and testis (D,E,I,J) cycles in the redheaded bunting and Indian weaverbird under natural (NDL, at Lucknow, India: 26°55' N, 80°59' E; 'A') and long (LD, 'F') photoperiods. In LD, birds were exposed first to weekly increments of 0.5 h light per day (with 0.25 h each in the morning and evening) for ten weeks, until short days (8 h light: 16 h darkness; LD 8:16) lengthened to long days (LD 13:11), and then to LD 13:11 for another 32 weeks. Data are plotted for individuals (scatter plot; B,D,G,I) as well as for groups (mean±S.E.; C,E,H,J).

*libitum*. The data are presented for individuals (scatter diagram) as well as for groups (mean±S.E.). To better illustrate the time course of the induction of a LHS, we calculated the time taken by individuals to show initiation, peak and regression in the response. The interval between the initiation and termination of a response gave the duration of the LHS. Thus, body mass gain–loss cycle was calculated as the interval between the time when buntings had gained 10% over the initial value and the time when body mass following peak again declined to the level of initial value. Similarly, testicular growth–regression cycle was calculated as the interval between the time of initiation of testis recrudescence and regression, using the following criterion: TV=0.33 to <2.35mm<sup>3</sup> = no response; 2.35 to <9.82mm<sup>3</sup> = initiation of response; TV=9.82 to <18.86mm<sup>3</sup> = small response; 18.86 to <41.9mm<sup>3</sup> = moderate response; 41.9mm<sup>3</sup> and above = full response (Kumar et al. 2002).

The data were analysed by one-way analysis of variance with repeated measures (one-way RM ANOVA), followed by Newman-Keuls *post hoc* test, to determine a significant change over the period of the experiment. We compared the response patterns between two species using two-way analysis of variance with repeated measures (mixed model; two-way RM ANOVA), followed by Bonferroni *post hoc* test, if ANOVA indicated a significant difference. Student's *t*-test compared data from two groups at the same time point. Significance was taken at  $P<0.05$ .

### 3. Results

Birds on NDL exhibited a significant change in their body mass over 47 weeks of the experiment [bunting:  $F(13,286)=17.22$ ,  $P<0.0001$ ; weaverbird:  $F(8,176)=9.158$ ,  $P<0.0001$ ; one-way RM ANOVA]. Buntings and weaverbirds did not differ in their response pattern, but exhibited a significant difference in time course of changes in their body mass [factor 1, species:  $F(1,462)=0.5040$ ,  $P=0.4856$ ; factor 2, time:  $F(22,462)=13.23$ ,  $P<0.0001$ ; interaction (species x time):  $F(22,462)=9.388$ ,  $P<0.0001$ ; two-way RM (mixed model) ANOVA; figures 1B, C and 2E].

Similarly, both species underwent significant testis recrudescence–regression cycles [buntings:  $F(13,143)=28.89$ ,  $P<0.0001$ ; weaverbirds:  $F(8,88)=56.85$ ,  $P<0.0001$ ; one-way RM ANOVA; cf. figure 1D and E], but with a significant difference between them [factor 1, species:  $F(1,231)=0.02709$ ,  $P=0.8708$ ; factor 2, time:  $F(11,231)=32.87$ ,  $P<0.0001$ , interaction (species x time):  $F(11,231)=23.18$ ,  $P<0.0001$ ; two-way RM (mixed model) ANOVA]. Overall, the timing and duration of body mass and testicular cycles was significantly different between two species (cf. figures 1B–E and 2A–D). As compared to weaverbirds, buntings had an earlier and shorter growth phase [buntings – 16.5±1.0 weeks;

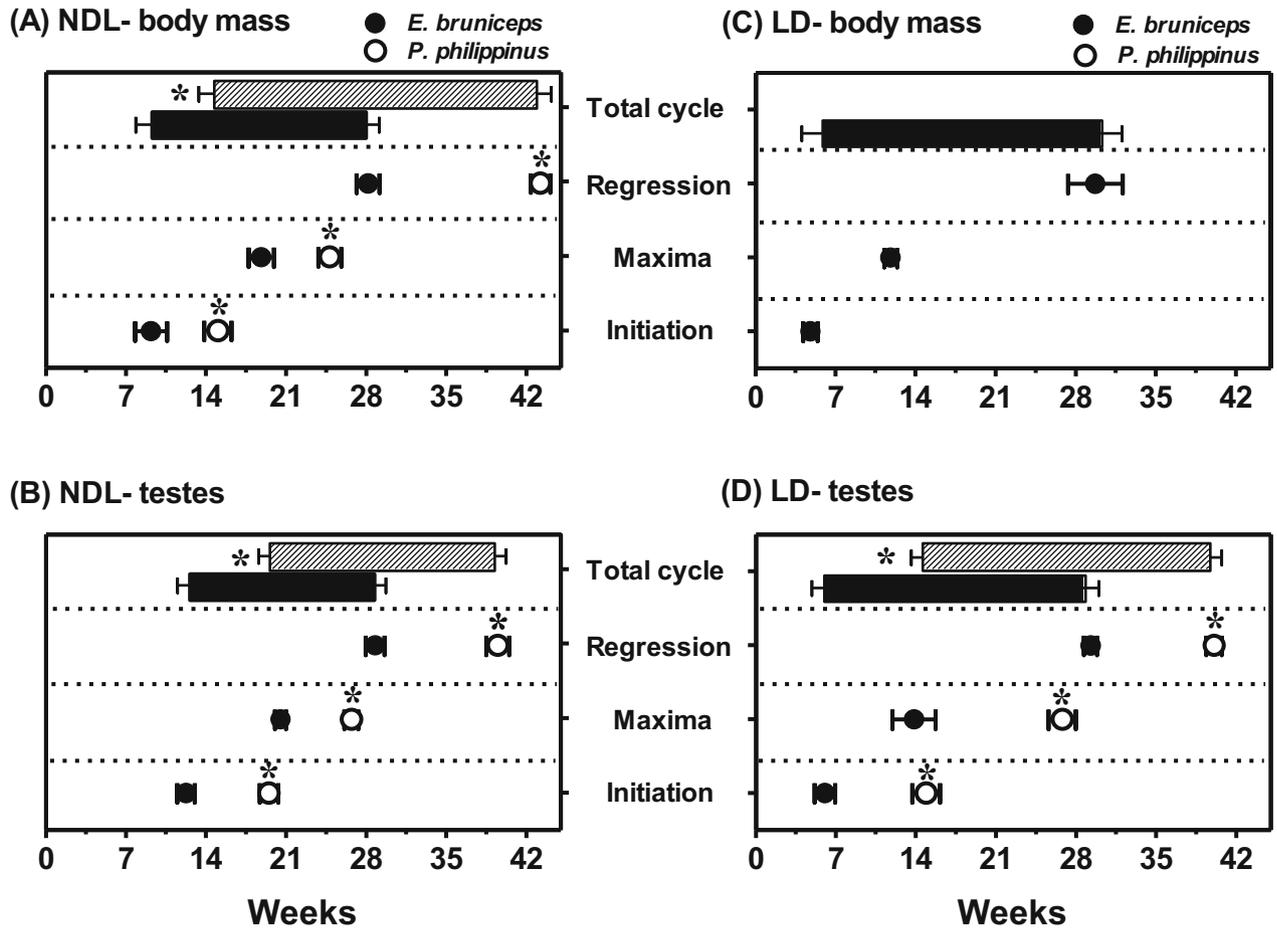
weaverbirds – 20.0±1.0 weeks;  $P<0.05$ , Student's *t*-test; cf. figures 1B–E and 2A–D].

On exposure to increasing and long photoperiods, both species exhibited similar growth–regression cycles in testes, but not in body mass (figure 1G–J). Whereas buntings showed a significant body weight gain–loss cycle of 24.9±2.4 weeks [ $F(6,47)=14.76$ ,  $P<0.0001$ ; one-way RM ANOVA; Neuman-Keuls *post hoc* test,  $P<0.0001$ ; figure 1G and H], weaverbirds lacked it although they exhibited a significant variation in body mass over the experimental period [ $F(6,47)=6.041$ ,  $P<0.0001$ , one-way RM ANOVA; figure 1G and H]. The gain in body mass in buntings was initiated in 4.8±0.6 weeks, reached peak in 11.8±0.6 weeks, and reduced again to almost initial values in 29.7±2.4 weeks (figures 1G, H and 2C). In spite of showing a similar gonadal cycle, the rate of testes maturation was significantly slower [ $P<0.05$ , unpaired Student's *t*-test] in weaverbirds than in buntings (figures 1I, J and 2D). Thus, testicular maturation cycle was delayed by 8.8±1.4 weeks in weaverbirds (cf. figures 1I, J and 2D). A comparison of long day induced body mass and testicular cycles also revealed significant difference between two species [body mass: factor 1, species –  $F(1,564)=14.06$ ,  $P=0.0028$ ; factor 2, time:  $F(47,564)=14.28$ ,  $P<0.0001$ ; interaction (species × time):  $F(47,564)=11.95$ ,  $P<0.0001$ ; testes: factor 1, species:  $F(1,204)=14.39$ ,  $P=0.0026$ ; factor 1, time:  $F(17,204)=27.08$ ,  $P<0.0001$ , interaction (species × time):  $F(17,204)=36.13$ ,  $P<0.0001$ ; two-way RM (mixed model) ANOVA]. The duration of reproductive LHS as defined by the interval between times of initiation of testicular recrudescence and regression was however similar in both species [bunting – 23.2±1.2 weeks; weaverbird – 24.2±0.6 weeks]. Further, the duration of testicular growth–regression cycle, as determined by the interval between times of initiation of testicular recrudescence and regression, was significantly longer in LD than in the NDL (bunting:  $P=0.0006$ ; weaverbird:  $P=0.0075$ ; Student's *t*-test).

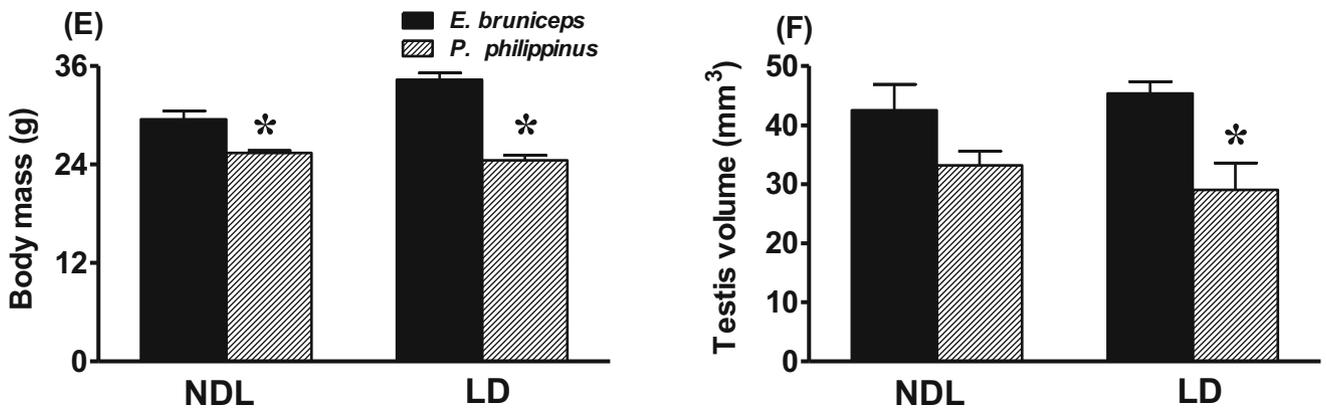
### 4. Discussion

Our results show that seasonal phenotypes were induced at significantly faster rates in the temperate redheaded bunting with five LHSs than in subtropical weaverbird with three LHSs in both LD and NDL groups (cf. figures 1 and 2). However, there was a significant difference in the testicular growth–regression cycle in between NDL and LD conditions; it was longer in the latter (figures 1 and 2). This could be due to the differences between two experimental conditions. The light and temperature conditions were constant under LD, while they along with other climatic conditions (e.g. humidity) significantly varied under NDL. More specifically, birds on NDL received much higher temperature (~40°C) during the period of May and June. High

### Duration of life history states



### Peak response



**Figure 2.** Life history states of the redheaded bunting and Indian weaverbird, derived from the data presented in figure 1. (A–D) represent (mean±S.E.) the timing and duration of life history states as indicated by symbols (open and solid circles) and bars (solid and hashed), respectively; (C) shows results from buntings only. (E) and (F) show maximum gain (peak response; mean±S.E.) in body mass and testis size under ND and long days, respectively.

temperature can shorten the duration of testicular growth, as has recently been reported in a study on blackheaded bunting (*Emberiza melanocephala*), which is a closely allied species of redheaded bunting. Blackheaded buntings exposed to stimulatory long days (LD 13:11) had significantly shorter testicular growth at high temperature (40°C) than at low temperature (27°C; Singh *et al.* 2012).

The present results are consistent with general patterns of reproduction and other LHSs reported among the temperate and tropical birds (Hau 2001). At high latitudes, birds appear to respond faster to photoperiods, in order to synchronize their activities in the year with highly seasonal environment (Martin 1996; Root 1988). This enables several species to adopt an expensive additional LHS, such as migration, in their annual itinerary. These temperate birds can also enter in an emergency life history state (ELHS) to overcome challenges posed by unpredictable changes in their climate conditions, such as sudden weather storms and temperature fluctuations (Wingfield and Kitaysky 2002; Wingfield *et al.* 1998). In contrast, birds in less seasonal tropical environments have more flexibility to adapt, thereby investing more on somatic maintenance (Martin 1996; Root 1988). Thus, tropical species with fewer LHSs have more temporal flexibility than temperate species with more LHSs in the year (Cardillo 2002; Wingfield 2008).

Avian annual itineraries are determined by the time constraints imposed upon them by the surrounding environment (Ricklefs 2000). These are arranged along a 'slow-fast' life-history axis, as a part of their adaptive strategy (Root 1988; Saether 1988). Tropical birds lie at the slow end of this continuum, as is indicated by a small clutch size (Cardillo 2002), slow nestling growth (Ricklefs 1976) and prolonged parental care (Russell *et al.* 2004). These traits lead low-latitude birds to have an increased fitness and higher rates of adult survival as compared to high-latitude species (Snow and Lill 1974; Wingfield 2008). One of the adaptations for slower pace of life in tropical birds is their reduced basal metabolic rate (Wiersma *et al.* 2007), as is evidenced by a study on the stonechat (*Saxicola torquata*). The sedentary tropical population of stonechats had reduced metabolic turnover and slower pace of LHSs as compared with their temperate migratory population (Wikelski *et al.* 2003).

In conclusion, the exposure of two songbird species to stimulatory photoperiods induced reproductive LHSs, which are similar to what they would possibly exhibit at their respective breeding latitudes. Further, the rates of induction of seasonal LHSs under long days were significantly faster in the temperate redheaded bunting than in the subtropical Indian weaverbird. This was regardless of the fact that for about a year the two songbird species closely shared their natural and experimental environments, and that buntings were held at a latitude (27°N) much lower than their breeding latitudes (~40°N). Thus, the present results extend the idea that along

with suites of life history traits in a species that are known to vary with latitude, there is a latitudinal effect on the plasticity of seasonal schedules that are regulated by environmental factors, e.g. day length. Could the present results also mean that avian annual itineraries, evolved from close interactions between the endogenous timing program and surrounding environment, are conserved at the species level among seasonally breeding birds? This will, however, need further investigations.

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### References

- Ali S and Ripley SD 1974 *Handbook of the birds of India and Pakistan*, vol. 10 (New York: Oxford University Press)
- Bradshaw WE and Holzapfel C 2007 Evolution of animal photoperiodism. *Annu. Rev. Ecol. Evol. Syst.* **38** 1–25
- Budki P, Rani S and Kumar V 2012 Persistence of circannual rhythms under constant periodic and aperiodic light conditions: sex differences and relationship with the external environment. *J. Exp. Biol.* **215** 3774–3785
- Cardillo M 2002 The life-history basis of latitudinal diversity gradients: how do species traits vary from the poles to the equator? *J. Anim. Ecol.* **71** 79–87
- Caro SP, Balthazart J, Thomas DW, Lacroix A, Chastel O and Lambrechts MM 2005 Endocrine correlates of the breeding asynchrony between two Corsican populations of blue tits (*Parus caeruleus*). *Gen. Comp. Endocrinol.* **140** 52–60
- Cooper CB, Margaret A, Voss MA, Ardia DR, Austin SH and Robinson WD 2011 Light increases the rate of embryonic development: implications for latitudinal trends in incubation period. *Funct. Ecol.* **25** 769–776
- Dawson A 2007 Seasonality in a temperate zone bird can be entrained by near equatorial photoperiods. *Proc. Royal Soc. Biol. Sci.* **274** 721–725
- Gwinner E 1996 Circadian and circannual programmes in avian migration. *J. Exp. Biol.* **199** 39–48
- Hau M 2001 Timing of breeding in variable environments: tropical birds as model systems. *Horm. Behav.* **40** 281–290
- Helm B, Schwabl I and Gwinner E 2009 Circannual basis of geographically distinct bird schedules. *J. Exp. Biol.* **212** 1259–1269
- Kumar V, Singh S, Misra M and Malik S 2001 Effects of duration and time of food availability on photoperiodic responses in the migratory male blackheaded bunting (*Emberiza melanocephala*). *J. Exp. Biol.* **204** 2843–2848
- Kumar V, Singh S, Misra M, Malik S and Rani S 2002 Role of melatonin in photoperiodic time measurement in the migratory redheaded bunting (*Emberiza bruniceps*) and the nonmigratory Indian weaver bird (*Ploceus philippinus*). *J. Exp. Zool.* **292** 277–286
- Martin TE 1996 Life-history evolution in tropical and south temperate birds: what do we really know? *J. Avian Biol.* **27** 1–10
- Perfito N, Meddle S, Tramontin AD, Sharp P and Wingfield JC 2005 Seasonal gonadal recrudescence in song sparrows:

- Response to temperature cues. *Gen. Comp. Endocrinol.* **143** 121–128
- Perfito N, Tramontin AD, Meddle S, Sharp P, Afik D, Gee J, Ishii S, Kikuchi M and Wingfield JC 2004 Reproductive development according to elevation in a seasonally breeding male songbird. *Oecologia* **140** 201–210
- Rani S, Singh S, Misra M, Malik S, Singh BP and Kumar V 2005 Daily light regulates seasonal responses in the migratory male redheaded bunting (*Emberiza bruniceps*). *J. Exp. Zool.* **303A** 541–550
- Rani S, Singh S and Kumar V 2007 Photoperiodism, pineal clock and seasonal reproduction in the Indian weaver bird (*Ploceus philippinus*). *J. Ornithol.* **148** 601–610
- Ricklefs RE 1976 Growth rates of birds in the humid new world tropics. *Ibis* **118** 179–207
- Ricklefs RE 1980 Geographical variation in clutch size among passerine birds: Ashmole's hypothesis. *Auk* **97** 38–49
- Ricklefs RE 2000 Density dependence, evolutionary optimization, and the diversification of avian life histories. *Condor* **102** 9–22
- Root T 1988 Energy constraints on avian distributions and abundances. *Ecology* **69** 330–339
- Russell EM, Yom-Tov Y and Geffen E 2004 Extended parental care and delayed dispersal: Northern, tropical, and southern passerines compared. *Behav. Ecol.* **15** 831–838
- Saether SA 1988 Pattern of covariation between life-history traits of European birds. *Nature* **331** 616–17
- Singh J, Budki P, Rani S and Kumar V 2012 Temperature alters the photoperiodically controlled phenologies linked with migration and reproduction in a night-migratory songbird. *Proc. Roy. Soc. B.* **279** 509–515
- Snow DW and Lill A 1974 Longevity records for some neotropical land birds. *Condor* **76** 262–267
- Wiersma P, Munoz-Garcia A, Walker A and Williams JB 2007 Tropical birds have a slow pace of life. *Proc. Natl. Acad. Sci. USA* **104** 9340–9345
- Wikelski M, Spinney L, Schelsky W, Scheuerlein A and Gwinner E 2003 Slow pace of life in tropical sedentary birds: a common-garden experiment on four stonechat populations from different latitudes. *Proc. R. Soc. Lond. B.* **270** 2383–2388
- Wingfield JC 2008 Organization of vertebrate annual cycles: implications for control mechanisms. *Phil. Trans. R. Soc. B.* **363** 425–441
- Wingfield JC and Kitaysky AS 2002 Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? *Integ. Comp. Biol.* **42** 600–609
- Wingfield JC, Breuner C, Jacobs J, Lynn S, Maney D, Ramenofsky M and Richardson R 1998 Ecological bases of hormone-behavior interactions: The 'emergency life history stage'. *Amer. Zool.* **38** 191–206

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