

## A circadian clock regulates migratory restlessness in the blackheaded bunting, *Emberiza melanocephala*

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Many diurnal songbirds undergo long-distance night-time migrations twice a year between their breeding and wintering grounds. When caged, this is reflected in intense night-time restlessness, which is called *Zugunruhe*. Whether migratory restlessness is regulated by the endogenous near-24 h (circadian) clocks is less understood. We investigated this in a Palearctic–Indian migratory songbird, the blackheaded bunting (*Emberiza melanocephala*), which in caged conditions exhibits *Zugunruhe*. Photosensitive birds ( $n = 8$ ) entrained to 8 h light: 16 h darkness (8L:16D) for 2 weeks were subjected to a stimulatory long day length (13L:11D; L = 450 lux, D = <1.0 lux); this resulted in intense night-time activity in all birds. After a total 7.5 weeks of 13L exposure, birds were released into constant dim light (LL<sub>dim</sub> = <1.0 lux; night-time illumination of LD) for 1 week. Movement of the bird within its cage (size = 60 × 45 × 35 cm) was continuously recorded using infrared sensors. The onset and end of activity at night-time were distinct from activity during daytime; ends consistently occurred about 1.5 h before the light on time. When released into LL<sub>dim</sub>, the buntings freeran with a circadian period with reference to activity at night-time. They also significantly lost their activity during daytime. These results suggest that with the onset of *Zugunruhe*, the circadian clock regulating daily activity rhythms redefines its phases, and so the birds become predominantly active at night.

**Keywords:** Bunting, circadian, oscillator, migration, *Zugunruhe*.

TWICE a year, many species of diurnal songbirds migrate at night several thousands of kilometres to reach their wintering grounds in late autumn and breeding grounds in late spring. In the non-migratory seasons, these birds are active during the day and inactive at night. In the migratory season, they remain active during the day but undergo a profound shift in their night-time behaviour: the birds fly at night<sup>1</sup>. When these birds are caged and thus disallowed from migrating, they exhibit intense restlessness at night reflecting the existence and intensity of their underlying physiological state. This is called migratory restlessness or *Zugunruhe*<sup>2</sup>, characterized by ‘wing whirring’ in several species<sup>3</sup>. It is shown in several long distance diurnal migra-

tory songbirds, including warblers (genus *Sylvia*)<sup>4</sup> that the temporal pattern of *Zugunruhe* faithfully reflects the temporal pattern of actual migration<sup>5</sup>.

Much research has been focused to understand how birds take a decision about when to begin flying, since it is critical for the successful completion of their annual journey<sup>1,6,7</sup>. Several studies establish that such decisions are based on a seasonal timing mechanism (circannual rhythm, *circa* = about; *annum* = year), which is similar to the mechanism that times daily occurrence of the daytime and night-time activities on a circadian (*circa* = about; *dies* = day) basis<sup>6</sup>. Like circadian rhythms, circannual rhythms are endogenously generated and synchronized by the environmental factors, viz. day length, weather conditions, food availability, etc.<sup>7</sup>. In many species, it is shown that exposure to long photoperiods induces the development of *Zugunruhe*<sup>8</sup>.

What controls the daily behavioural shift in a nocturnal migrant from inactivity at night in the non-migratory season to intense activity at night in the migratory season is less understood. We propose that with the onset of a migratory season, the circadian system controlling daily activity in a diurnal migrant redefines itself. Then the prediction is that with the onset of migration there would be dramatic behavioural shift, and if birds showing *Zugunruhe* were transferred to constant conditions, they would exhibit circadian rhythm in activity with reference to the onset and end of night-time. We investigated this using a long-distance migratory songbird, the blackheaded bunting (*Emberiza melanocephala*). This emberizid finch arrives in India in October/November, overwinters around 25°N, and returns to its breeding grounds located in West Asia and East Europe at ~40°N and higher; the bird covers a distance of about 7000 km during its migration<sup>9</sup>. Also, it is a photosensitive species and increasing day lengths of late March/April (≥ 12 h light per day) stimulate body fattening and *Zugunruhe* at night<sup>10</sup>.

The experiment was conducted on adult male blackheaded bunting, which were caught from the wintering flock in late February 2002 and placed first in the outdoor aviary before being moved indoors in March 2002 on 8 h light: 16 h darkness (8L:16D; L = 450 lux, D = <1.0 lux) until the beginning of the experiment. The activity of a bird within its cage was considered reflecting the response of its circadian system. Eight buntings were individually housed in specially designed activity cages (size = 60 × 45 × 35 cm) that were placed singly in photoperiodic chambers (size = 71 × 67 × 48 cm) providing light–dark cycles (L = 450 lux, D = <1.0 lux). First, the birds were exposed to 8L:16D for 2 weeks, and then to 13L:11D for 7.5 weeks. At the end of 13L exposure, they were released into dim constant light (LL<sub>dim</sub> = <1.0 lux night-time illumination) for 1 week by turning off the lamp, providing daytime light to determine the phase and period of the endogenous circadian clock. Food and water were available *ad libitum*.

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The activity rhythm defined by the record of movement of a bird within its cage detected by a Passive Infrared Motion Sensor was plotted as a double plot, as earlier described for this species<sup>11</sup>. A continuous recording and analyses of activity were done using the program supplied by Stanford Software Systems (Stanford, USA). The onset and end of activity during daytime was calculated with reference to periods of 'light on' and 'light off' respectively. Similarly, the onset and end of activity at night-time was calculated with reference to period of 'light off' and 'light on' respectively. The phase relationships between activity and LD cycle were described as phase angle difference ( $\psi$ ). We calculated the period of the rhythm using chi-square periodogram analysis program of Stanford Software Systems. Data on activity profile and circadian period ( $\tau$ ) are presented as means  $\pm$  SE and when appropriate, they were compared using Student's *t*-test. Significance was taken at  $P < 0.05$ .

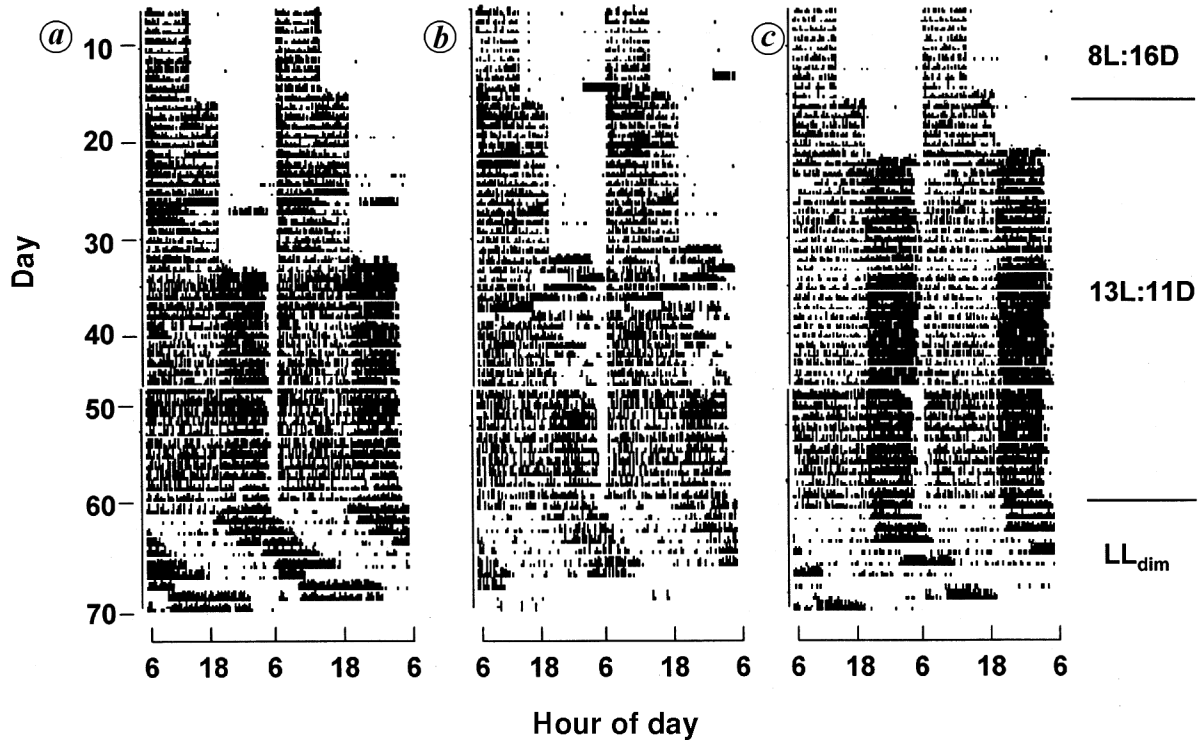
The results are shown in the Figures 1 and 2. Birds were entrained to 'light on' of the LD cycle. Under 13L:11D, activity was restricted to light phase, and the onset and end of daytime activity were locked onto 'light on' and 'light off' respectively ( $\psi$  onset =  $-0.03 \pm 0.03$  h;  $\psi$  end =  $0.00 \pm 0.04$  h). After several days of 13L exposure, there occurred a behavioural shift (Figure 1 a-c); intense activity developed at night along with activity during daytime. The timing of the development of night activity varied among individuals: whereas in most birds it developed in about 2 to 2.5 weeks, in two individuals it developed by the end of the first week of a long day. In all birds, however, the activity at night began immediately after 'light off' ( $\psi$  onset =  $-0.36 \pm 0.05$  h) and ended well before 'light on' ( $\psi$  end =  $1.64 \pm 0.27$  h); onset of activity was more precise than end of activity. The end of night-time activity was spaced from the beginning of daytime activity by about  $1.67 \pm 0.28$  h, but this interval was reduced to  $0.37 \pm 0.07$  h between the beginning of the night-time activity and the end of daytime activity. The amount of activity at night was, however, not significantly different from that during daytime (mean  $\pm$  SE) counts for 12 days preceding transfer to LL<sub>dim</sub>: daytime =  $1378.39 \pm 233.74$ ; night-time =  $1498.45 \pm 276.52$ ). Average activity per hour at night ( $115.27 \pm 21.27$ ) was also not significantly different ( $P = 0.7453$ , Student's *t*-test) from the average activity per hour during daytime ( $106.03 \pm 17.98$ ). When released into LL<sub>dim</sub>, birds started showing endogenous periods of their circadian clock with reference to night-time activity (Figure 1);  $\tau$  equalled  $25.77 \pm 0.15$  h. Also, there was a significant loss ( $P < 0.001$ , Student's *t*-test) of activity during the daytime (cf. Figure 2).

The present study shows that the blackheaded bunting undergoes a dramatic behavioural shift from being exclusively day-active to both day- and night-active (Figure 1 a-c; cf. activity under LD before and after the development of *Zugunruhe*). A difference in the timing of the development of *Zugunruhe* among eight individuals in the

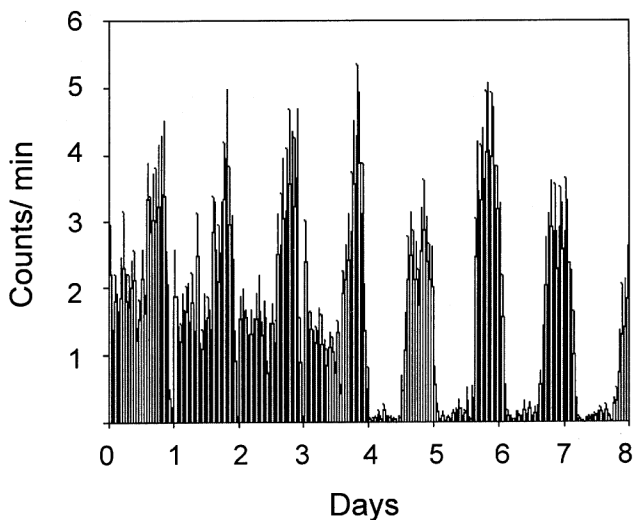
experiment (cf. Figure 1 c) indicated that the onset of spring migration of a population from its wintering territories was spread over several days. A few species of birds<sup>6,12</sup>, fish<sup>13</sup> and rodents<sup>14</sup> have also been reported, showing behavioural shift. In the present study, a dramatic behavioural shift (Figure 1 a-c) under long days suggests one or both of the following possibilities. First, with the onset of nocturnal migration, the circadian clock regulating daily activity rhythm redefines its phases: birds become inactive during daytime and intensely active at night. If that were the case, the activity of buntings during daytime after the development of *Zugunruhe* represents the light-induced masked activity. This was confirmed by comparing activity profiles before and after transfer to LL<sub>dim</sub>. Plot of activity profile over a four-day period before and after release into LL<sub>dim</sub> (Figure 2) clearly shows that this was indeed the case, since activity during the subjective daytime of LL<sub>dim</sub> condition was significantly reduced ( $P < 0.001$ ) compared to activity of the same birds during daytime in the preceding LD condition; however, there was no reduction in night-time activity.

A second possibility is that separate circadian oscillators control daytime and night-time activities in the black-headed bunting. *Zugunruhe* results from the interactions of these two (or more) circadian oscillators; and the phase relationship between them determines the timing of the onset of *Zugunruhe*. A recent study on migratory garden warblers (*Sylvia borin*)<sup>15</sup> supports this. If that were the case, in non-migratory season both the daytime and night-time circadian oscillators are tightly coupled with an overlapping phase, such that activity is expressed only during daytime. In the migratory season, these oscillators uncouple and their changed phase relationship results into the development of *Zugunruhe*. This, however, does not explain the loss of activity during the subjective daytime under LL<sub>dim</sub> in the present study (Figures 1 a-c and 2) unless we assume the dominance of one of the two oscillators in controlling the activity of the locomotor centre within the brain of the bird. This needs to be investigated.

The idea that separate oscillators control the day-time and night-time activity components in migrants exists in the literature<sup>6,7,12</sup>. Gwinner<sup>12</sup>, based on his studies on migratory European robin (*Erithacus rubecula*), suggested day and night activity components as being controlled by two coupled oscillators that were dependent on contrasting light intensities (high intensity – day-time activity; low intensity – night-time activity). Pohl<sup>16</sup> further showed evidence of different oscillators controlling day-time activity and night-time migratory restlessness in the brambling (*Fringilla montifringilla*). All this is also consistent with the phenomenon of splitting of circadian rhythms in activity found in several species, which is considered reflecting change in phase relationships of the underlying oscillators<sup>17</sup>. Given the evidence that the splitting of circadian activity rhythms in European starling (*Sturnus vulgaris*) can be induced by exogenous administration of testoster-



**Figure 1.** *a–c*, Double plotted activity recordings of three representative blackheaded buntings exposed first to 8 h light : 16 h of darkness (8L : 16D; 0600–1400 h; L = 450 lux, D = <1.0 lux) for 2 weeks and then 13L : 11D (0600–1900 h; L = 450 lux, D = <1.0 lux) for 7.5 weeks before being released into constant dim light ( $LL_{dim}$  = <1.0 lux; nighttime illumination of the LD) for 1 week. Movement of the bird within its cage (size = 60 × 45 × 35 cm) was detected by Passive Infrared Motion Sensor, and continuously recorded and analysed by the software program of Stanford Software Systems (Stanford, USA). Between days 40 and 50, activity recordings for 3 days were lost due to technical error.



**Figure 2.** Mean ( $\pm$ SE,  $N = 8$ ) activity profile presented as counts per minute for four cycles before and after transfer to  $LL_{dim}$ . Note the significant loss ( $P < 0.05$ ) of activity during the subjective day-time under  $LL_{dim}$ .

one<sup>18</sup>, it is logical to propose that hormonal changes that occur seasonally in a migratory bird induce change in the phase relationship between underlying circadian oscillators

with the onset of the new season. Spontaneous changes in the characteristics of a circadian rhythm or in the phase relationship between different circadian rhythms are known in several birds<sup>19,20</sup>.

In conclusion, the present results suggest that in diurnal migrants with the onset of the *Zugunruhe*, the circadian clock regulating daily activity rhythm redefines its phases, so that there is a behavioural shift from being day-active to predominantly night-active.

1. Berthold, P., *Control of Bird Migration*, Chapman and Hall, London, 1996.
2. Wagner, H. O., Über Jahres- und Tagesrhythmus bei Zugvögeln. *Z. Vgl. Physiol.*, 1930, **12**, 703–724.
3. Berthold, P. and Querner, U., Was Zugunruhe wirklich ist: Eine quantitative Bestimmung mit Hilfe von Video-Aufnahmen bei Infrarotbeleuchtung. *J. Ornithol.*, 1988, **129**, 372–375.
4. Gwinner, E., Adaptive functions of circannual rhythms in warblers. In Proc. 15th Int. Ornithol. Congr., 1972, pp. 218–236.
5. Gwinner, E., Circannual rhythms in the control of avian migrations. *Adv. Stud. Behav.*, 1986, **16**, 191–228.
6. Gwinner, E., Circadian and circannual programmes in avian migration. *J. Exp. Biol.*, 1996, **199**, 39–48.
7. Gwinner, E. and Helm, B., Circannual and circadian contributions to the timing of avian migration. In *Avian Migration* (eds Berthold, P., Gwinner, E. and Sonnenschein, F.), Springer, Berlin, 2003, pp. 81–95.

8. Gwinner, E., Circannual systems. In *Handbook of Behavioral Neurobiology* (ed. Aschoff, J.), Plenum Press, New York, 1981, pp. 391–410.
9. Ali, S. and Ripley, S. D., *Handbook of Birds of India and Pakistan*, Oxford University Press, Bombay, 1974, vol. 10.
10. Jain, N., Strategies for endogenous programming in the migratory blackheaded bunting, *Emberiza melanocephala* Scopoli. Ph D thesis, Meerut University, India, 1993.
11. Malik, S., Rani, S. and Kumar, V., Wavelength dependency of light-induced effects on photoperiodic clock in the migratory blackheaded bunting (*Emberiza melanocephala*). *Chronobiol. Int.*, 2004, **21**, 367–384.
12. Gwinner, E., Circadian and circannual rhythms in birds. In *Avian Biology* (eds Farner, D. S. and King, J. R.), Academic Press, New York, 1975, vol. 5, pp. 221–285.
13. Müller, K., Seasonal phase shift and the duration of activity time in the burbot, *Lota lota* (L.) (Pisces, Gadidae). *J. Comp. Physiol.*, 1973, **84**, 357–359.
14. Erkinaro, E., Seasonal changes in the phase position of circadian activity rhythms in some voles and their endogenous component. *Aquilo Ser Zool.*, 1972, **13**, 87–91.
15. Bartell, P. A. and Gwinner, E., A separate circadian oscillator controls nocturnal migratory restlessness in the songbird *Sylvia borin*. *J. Biol. Rhythms*, 2005, **20**, 538–549.
16. Pohl, H., Circadian control of migratory restlessness and the effects of exogenous melatonin in the brambling, *Fringilla montifringilla*. *Chronobiol. Int.*, 2000, **17**, 471–488.
17. Pittendrigh, C. S. and Daan, S., A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: a clock for all seasons. *J. Comp. Physiol.*, 1976, **106**, 333–355.
18. Gwinner, E., Testosterone induces ‘splitting’ of circadian locomotor activity rhythms in birds. *Science*, 1974, **185**, 72–74.
19. Eskin, A., Some properties of the system controlling the circadian activity rhythm of sparrows. In *Biochronometry* (ed. Menaker, M.), National Academy of Sciences, Washington, DC, 1971.
20. Aschoff, J. and Pohl, H., Phase relations between a circadian rhythm and its zeitgeber within the range of entrainment. *Naturwissenschaften*, 1978, **65**, 80–84.

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## Foraminiferal biostratigraphy of the Early Cretaceous Hundiri Formation, lower Shyok area, eastern Karakoram, India

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**The arc sequence in the lower Shyok area comprises volcanics (Shyok Volcanics) and intra-arc basin sediments (Hundiri, Saltoro Flysch and Saltoro Molasse forma-**

**tions). Biostratigraphic investigations on the Hundiri Formation indicate that this lithounit contains several Aptian–early Albian orbitolinids comprising *Mesorbitolina*, *Orbitolina*, *Praeorbitolina*, *Palorbitolinoides*, *Palorbitolina*, *?Neorbitolinopsis* and *Paracoskinolina*. The orbitolinid taxa from the Shyok tectonic belt are comparable with those known to occur in Ladakh, Chitral, Burzil Pass (Indo-Pak region), Afghanistan, South Tibet and Myanmar. Fossil data suggest the existence of transgressive Neo-Tethys sea during Early Cretaceous, north of the Indian Plate.**

**Keywords:** Aptian–Albian, orbitolinids, biostratigraphy, eastern Karakoram.

THE Shyok tectonic belt, which lies in the northwestern part of Ladakh, is a geologically poorly known terrain (Figure 1a, b). In this belt several igneous, sedimentary and metamorphic rock types occur and are sandwiched between the Ladakh Batholith to the south and the Karakoram Batholith to the north (Table 1). The marine sedimentary succession in the lower Shyok area is represented by the Hundiri and Saltoro Flysch formations. The latter lithounit is overlain unconformably by the Saltoro Molasse Formation. The Shyok tectonic belt attracted the attention of several pioneering geologists<sup>1,2</sup>. This region is of great geological interest as it is related to subduction of the Indian Plate below the southern margin of Asia and was studied by several researchers in the recent past<sup>3–9</sup>. The Hundiri and Saltoro Flysch formations (=Saltoro Formation of Upadhyay<sup>8</sup>) (Figure 2) are well exposed at several localities in the Saltoro Hills and are also exposed along the right bank of Shyok river (Figure 1b). Sections of the Hundiri Formation from east to west were observed along the Dosam–Biagdong route at several localities, namely Dosam (a camp site east of Hundiri village), Hundiri, Hora Sostan villages and Sukur Nala. The outcrop of this formation extends up to Biagdong and further west.

Orbitolinids are larger foraminifera that are helpful for stratigraphic calibration. They belong to the family Orbitolinidae – an important fossil group, known to occur in the pelagic carbonate platform facies – and range from Barremian to Cenomanian (middle part of the Cretaceous, i.e. approx. 125–93.5 m.y.). Thus, orbitolinids are restricted to a short stratigraphic range and have a wide geographical distribution. They are helpful in palaeogeographic reconstruction and in interpreting environment of deposition. They are well developed in carbonate build-ups, volcanic seamounts in the Shyok Suture, Ladakh, India and are excellent markers in correlation on a global scale<sup>10</sup>. Along this suture, the Ladakh arc sediments containing orbitolinids indicate transgression of sea during Aptian–Albian. Appearance of orbitolinid taxa in Shyok suture zone indicates that the sea transgressed in this zone during early Cretaceous time. In the Indian subcontinent, coeval orbitolinid taxa occur in the Lhasa–Shyok–Kohistan block as well as in the Indus suture zone of Ladakh, indicating the presence of transgressive sea in these areas during Aptian–Albian.

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