

## Persistence of circannual cycles in a migratory bird held in constant dim light

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Accepted July 31, 1992

**Summary.** 1. Most studies of long-term endogenous cycles in birds have been performed in light:dark (L:D) cycles. In this study, male dark-eyed juncos (*Junco hyemalis*) were held in constant dim light (1–3 lx) and constant temperature for up to 3 years. Testicular cycles, molt, migratory fattening and nocturnal migratory activity (*Zugunruhe*) were monitored continuously.

2. Birds exhibited up to 3 cycles of gonadal growth and regression, spring pre-migratory fattening, molt, and spring and fall *Zugunruhe*. The annual cycle events maintained appropriate sequential relationships. The amplitude of successive testicular cycles did not decrease.

3. The annual cycles of individual birds drifted out of phase with one another, i.e., they appeared to free-run. Cycle lengths were quite variable: 6 to 20 months based on the interval between testicular maxima, and from 6 to 21 months based on the interval between the onset of post-nuptial molts. The cycles had a mean duration of 13.7 months (SD=4.2 months) between testicular maxima, and 15.0 months (SD=3.8 months) between the onset of post-nuptial molts. Therefore they may be considered circannual.

4. These data provide the strongest evidence for the existence of endogenous, free-running long-term rhythms in birds. They are consistent with a hypothesis in which annual cycles are based on a self-sustaining circannual oscillator upon which seasonal changes in daylength act as a *Zeitgeber*.

**Key words:** Circannual rhythms – Annual cycle – Internal coincidence – Endogenous rhythms – *Junco hyemalis*

### Introduction

Many bird species live most of their lives in regions where changes in daylength provide seasonal cues for the timing

*Abbreviations:* AHY, after hatching year; D, dark; HY, hatching year; L, light

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of reproduction, molt and migration. The earliest empirical demonstrations of the positive effect of increasing daylength on the reproductive state of birds were performed on a North American migrant, the dark-eyed junco (*Junco hyemalis*) (Rowan 1925). Since then, the influence of changing photoperiod on the events of the annual cycle has been observed in many species of animals and plants. During the last 25 years, long-term studies on a variety of animal taxa have concluded that the annual cycle is controlled by a free-running, endogenous rhythm [reviewed in Gwinner (1986)]. In this scheme, changes in photoperiod do not drive the events of the annual cycle, but serve simply as a *Zeitgeber* that entrains the putative self-sustaining circannual oscillator to the external seasonal cycle. Most of the studies of circannual rhythms in birds have been performed in constant L:D photoregimes (e.g., 12L:12D). Under these conditions, many species exhibit repeated gonadal cycles, molt, periods of migratory activity, etc., for up to 10 years (Gwinner 1986). Especially in studies on the European starling (*Sturnus vulgaris*), it has been found that there is a very narrow range of “permissive photoperiods”, daylengths under which repeated, apparently free-running cycles persist (Schwab 1971; Gwinner 1975, 1981; Gwinner et al. 1985). The permissive photoperiods vary across species in ways that are believed to reflect adaptations to the different photoperiodic regimes experienced over the course of a year (Gwinner 1989). In fact, Gwinner (1990) has suggested that certain photoperiodic conditions may have to be met once or several times per cycle in order for the underlying rhythm to persist.

The apparent strong dependence on photoperiodic conditions of repeated circannual cycles, and the fact that few conclusive experiments have been performed in the absence of a L:D cycle, causes many to remain skeptical of the existence of long-term endogenous rhythms in birds (King 1968; Hamner 1971; Rutledge 1974; Sansum and King 1976; Farner and Follett 1979; Wingfield and Farner 1980; Donham et al. 1983; Farner 1985; Nicholls et al. 1988). Although Aschoff (1980) and

Gwinner (1986) provided elegant theoretical rebuttals to these arguments, there remains a need for strong empirical evidence for the occurrence and persistence of free-running annual cycles in birds in the absence of a L:D cycle.

This study sought to determine whether the dark-eyed junco, a North American migratory bird, would exhibit repeated, free-running annual cycles in constant dim light. We monitored indicators of the major events of the annual cycle of this species (*Zugunruhe*, migratory fattening, gonadal recrudescence and regression, molt) in males held under constant environmental conditions for up to 3 years.

### Materials and methods

Dark-eyed juncos were captured in mist nets at Albany, New York. Sex of the birds was determined by laparotomy. Age of the birds (hatching year = HY; after hatching year = AHY) was determined by degree of skull ossification and plumage characteristics (Grant and Quay 1970). We placed 20 males in an environmental chamber on 16 November 1987, and maintained them in constant dim light (L:L, 1–3 lx) and constant temperature ( $20 \pm 2^\circ\text{C}$ ). The birds were visually, but not acoustically, isolated from one another. Birds that died during the experiments were replaced with new individuals. Loud circulation fans prevented sounds from outside the chamber to be heard inside. Food (mix of turkey starter and millet) and water were given ad libitum and replaced every 1–3 days on a variable schedule to prevent our activity from acting as a possible entrainment cue. To assess the state of the left testis, we performed a laparotomy on each bird approximately every 4–6 weeks, beginning in February, 1988. All procedures were performed in the experimental chamber in the ambient dim light. The length of the left testis was estimated with the aid of a point light source while the bird's head was covered with an opaque black bag to prevent any direct illumination of the head region. The flight feathers of the wings and tail were clipped so that molt could be easily detected at each examination. The amount of visible subcutaneous fat was also noted (scoring based on Cherry 1982). We recorded perch hopping activity continuously with an Esterline-Angus event recorder.

**Table 1.** Details of the annual cycles of juncos held for up to 37 months in constant dim light (L:L).<sup>1</sup> Age is denoted as HY (hatching year) for birds placed in L:L when less than 1 year old, or AHY (after hatching year) for birds older than 1 year. A testicular cycle comprised the full recrudescence (to  $\geq 6$  mm length) of the regressed testis while in L:L. Interval between testicular

We estimated the length of an individual's annual cycle by two criteria: (1) we counted the number of months between the onset of successive post-nuptial molts; and (2) we counted the number of months between successive maxima in testicular size (a maximum was defined as the time at which laparotomy showed that the recrudescing testis reached  $\geq 6$  mm in length, a size at which the testis is actively producing sperm and hormones; Wolfson 1952). These two measures of an annual cycle length are probably not independent but they were chosen, a priori, in case cycle events became dissociated under free-running conditions.

### Results

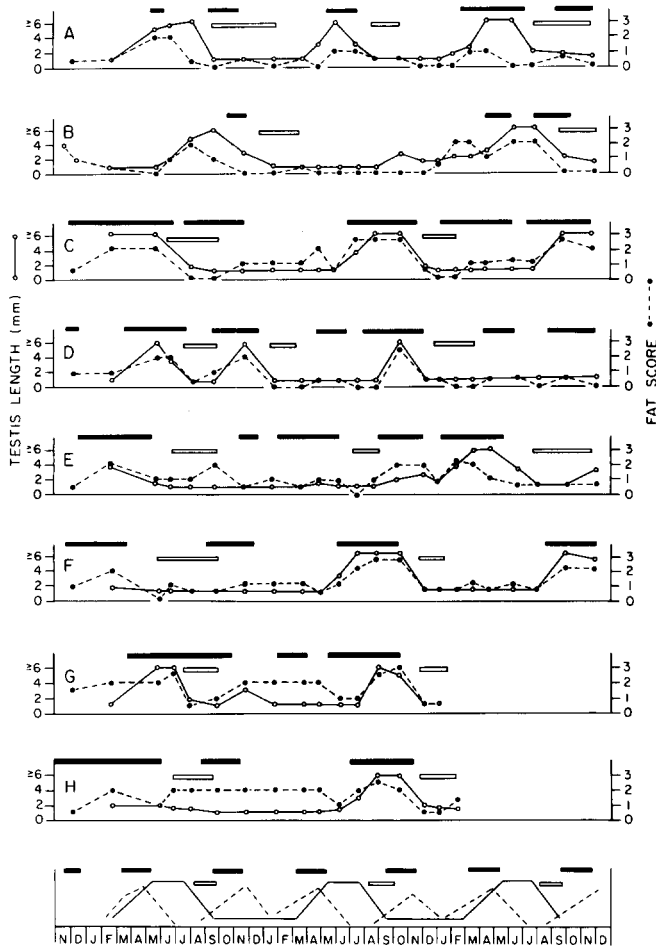
Data on the birds used in the experiment, the length of time they were held in L:L, and the number of annual cycles undergone by each are shown in Table 1. Of the birds (22) that spent at least one year in L:L, only one failed to show a testicular recrudescence. Six of the birds that began the experiment in November, 1987, survived until it ended unexpectedly in November, 1990. Figure 1 shows the data from the 8 birds that completed at least one testicular or molt cycle in L:L. They are typical of the other birds that were maintained in L:L for shorter periods of time. These records illustrate several important points: (1) Although housed individually in cages within the same environmental chamber, the annual cycles of the birds drifted out of phase with one another i.e., they showed free-running behavior. (2) All of the annual cycle events that we monitored occurred repeatedly and maintained appropriate sequential relationships, viz., spring pre-migratory fattening, spring *Zugunruhe*, gonadal growth and regression followed by post-nuptial molt and fall *Zugunruhe*. (3) The amplitude of successive testicular cycles showed no indication of decrease. Mean values based on the interval between testicular maxima ( $\bar{x} \pm \text{SD}$ ,  $13.7 \pm 4.2$  months;  $n=6$ ) and that between the onset of successive post-nuptial molts ( $\bar{x} \pm \text{SD}$ ,  $15.0 \pm 3.8$  months;  $n=10$ ) were similar and in both cases slightly

maxima was the time between the first measurement in each cycle at which the left testis was  $\geq 6$  mm. To compute mean annual cycle lengths for all birds combined, we have used one value per individual; for birds with more than one measured interval, we have used the mean of the intervals (shown in parentheses)

Age	Date into L:L (mo./yr.)	Months in L:L	Number of cycles		Interval (months) between		Fig.
			Testicular	Molt	Testicular maxima	Onset of molt	
HY	11/87	37	3	3	10, 10 (10.0)	11, 12 (11.5)	1A
AHY	11/87	37	2	2	20	21	1B
AHY	11/87	37	3	2	18, 12 (15.0)	18	1C
HY	11/87	37	3	3	6, 10 (8.0)	6, 11 (8.5)	1D
AHY	11/87	37	1 <sup>2</sup>	3	–	13, 12 (12.5)	1E
AHY	11/87	37	2	2	14	18	1F
HY	11/87	25	2	2	15	16	1G
AHY	11/87	27	1	2	–	18	1H
AHY	06/89	16	1	2	–	13	–
AHY	05/89	16	1	2	–	13	–

<sup>1</sup> Twelve additional birds were held in L:L for 10–24 months. All but one showed one testicular cycle and all had one post-nuptial molt while in L:L.

<sup>2</sup> Beginning second testicular recrudescence at termination of experiment (see Fig. 1E).



**Fig. 1A-H.** Annual cycles of 8 male dark-eyed juncos housed in constant dim light and constant temperature for 3 years. *Solid lines* = testis length; *broken lines* = fat score; *solid bars* = periods of nocturnal migratory activity; *open bars* = periods of molt. A hypothetical example of the sequence of annual cycle events expected in a junco under natural photoperiod is presented at the bottom for comparison

greater than 12 months. Thus the periodicity can be considered circannual (Gwinner 1986), but both inter- and intra-individual variation in cycle length were considerable (Table 1).

The testes of these males showed cycles of recrudescence and regression that were typical of juncos and other emberizines held in captivity under natural photoperiodic conditions (Wolfson 1952; Sansum and King 1976; Donham et al. 1983). In fact, in terms of the maximal testicular sizes achieved by the birds and the duration of testicular maxima ( $x \pm SD$ ,  $2.4 \pm 0.5$  months), the birds were similar to captive juncos maintained under natural photoperiod (Wolfson 1952) and free-living emberizines of similar size (Wingfield 1984). During recrudescence, the testes exhibited pronounced surface blood vessels and the birds had well-developed cloacal protuberances. Vernal pre-migratory fattening occurred in nearly all of the birds, but most did not deposit substantial fat reserves after post-nuptial molt and associated with fall migration. In the latter regard, the birds in L:L differed from captive juncos under natural

photoperiod (Holberton 1991; cf Wolfson 1952) and from free-living birds. As is typical of nocturnal migrants held in captivity (e.g. Ketterson and Nolan 1985), birds remained fat and *Zugunruhe* persisted into what would have been the breeding season. Subcutaneous fat deposits generally decreased coincident with testicular regression.

## Discussion

The data presented here provide the strongest evidence from birds that repeated cycles of annual events occur and free-run with a periodicity of approximately 12 months under constant conditions. Although free-running circannual rhythms have been well-documented in ground squirrels (*Spermophilus* spp.) (Pengelley et al. 1976; Davis and Swade 1983), surprisingly few studies have been done with birds maintained in L:L or D:D. Pekin ducks held from 4 to 6 years in both L:L and D:D showed repeated cycles of testicular growth and regression but with periods of 5–7 months that cannot be considered circannual (Benoit et al. 1970; Gwinner 1986). Sansum and King (1976) held white-crowned sparrows (*Zonotrichia leucophrys gambelii*) under two constant photoperiods and L:L for up to 2.5 years. Most of the birds were placed into L:L during or immediately prior to testicular recrudescence; none showed a second recrudescence. The light intensity was high during this experiment (450–500 lx) and may have induced photorefractoriness (a “long day” response). At the end of the experiment, the L:L birds became photosensitive after exposure to “short” days (8L:16D) for 6 weeks.

Chandola et al. (1982) held the non-migratory, tropical spotted munia (*Lonchura punctulata*) in L:L of 300 lx for 32 months. The birds (all males) were held in flock cages and went through 3 testicular cycles of ca. 10 months. The cycles of the 15 birds in the flock remained synchronized, perhaps by social stimuli as suggested by the authors. More puzzling, the cycles of the birds in L:L were remarkably synchronous with those of another group housed in 12L:12D in a separate room. The absence of clear evidence of free-running in the records from these munia raises the possibility that the birds were entrained by some non-photoc environmental cue. Later, Bhatt and Chandola (1985) held male spotted munia in flock cages for 29 months in L:L of 90 lx. The birds showed two cycles of testicular recrudescence/regression and two cycles of body weight change with periods of about 10 months. Although no evidence was presented that the individual birds exhibited free-running annual cycles, and the testes of the L:L males reached only about one-half the volume of birds on natural photoperiod, these data represented the strongest case for the persistence of avian circannual cycles under constant conditions. The variability in cycling behavior observed within species and the small number of species studied to date suggest caution concerning the generality and robustness of long-term endogenous cycles.

We deliberately chose to use light of an intensity too dim to be photoinductive (Bartholomew 1949) or to

induce circadian arrhythmicity, a frequent occurrence when birds are placed in L:L of  $> 10$  lx (McMillan et al. 1975). Under these conditions, at least in the dark-eyed junco, all of the components of a typical annual cycle recurred spontaneously. Apparently entirely endogenous changes are sufficient to induce all of the physiological transitions between successive stages. When present, L:D cycles have potent effects on the expression of circannual rhythms. Cycle lengths tend to be less variable and generally shorter than those we observed under L:L (Berthold et al. 1972; Gwinner 1981, 1986). Furthermore, in the European starling, e.g., it has been found that there is a narrow range of "permissive photoperiods", daylengths under which repeated, apparently free-running circannual cycles persist. Starlings held on 11L:13D or 13L:11D do not exhibit repeated cycles, whereas on 12L:12D they do (Schwab 1971; Gwinner et al. 1985). The underlying mechanism is apparently that days of 11 h are not long enough to induce gonadal regression: once recrudesced, the birds' gonads remain active indefinitely. Thirteen-hour days are too long to break photorefractoriness, so the gonads remain quiescent.

By definition, photorefractoriness is a response to photoperiod. The accompanying physiological states (rapid gonadal regression, loss of responsiveness to daylengths that are stimulatory at other times) seem at least partly to be due to a cessation of GnRH production in the hypothalamus, which also contains photoreceptors involved in the measurement of daylength (Nicholls et al. 1988; Homma et al. 1980). Because our birds were maintained in constant dim light, we do not know how they would have responded to "long" days at various times during the annual cycle. It is clear, however, that the gonads of these birds regressed spontaneously and subsequently became active again. This suggests that the internal changes associated with photorefractoriness (e.g., those in the hypothalamus) may occur in the absence of a L:D cycle.

Although the biological basis of circannual rhythms and how they may be linked to circadian rhythms are unknown, our data support Aschoff's (1955) model wherein annual cycles are based on a self-sustaining endogenous oscillator upon which changes in daylength act as a *Zeitgeber*, rather than as a driver of the annual cycle. Gwinner (1973) proposed a modified internal coincidence model (Pittendrigh 1972) in which circannual rhythms may be derived from changing phase relationships between two circadian oscillators that may occur in the absence of a L:D cycle. Meier and his colleagues (reviewed in Meier and Russo 1985) have developed a specific hypothesis for the control of annual cycles based on changing phase relationships between circadian rhythms in circulating levels of prolactin and corticosterone. Our results suggest that it would be productive to study endogenous hormone rhythms in birds held long term under constant conditions, so that we will be able to distinguish those hormonal events that are spontaneous and essential for the progression of annual cycle events from those which may be driven by photoperiod.

*Acknowledgements.* We thank Barbara Braeker and Edda Schmidt for their help in caring for the birds, and John McCormick for his efforts to keep the chamber running properly. This work was supported by the National Science Foundation (grants BNS 8608653 and BNS 8909886 to K.P. Able) and by grants from Sigma-Xi and the SUNY Benevolent Fund to R.L. Holberton. The work was performed in accordance with guidelines of the Institutional Animal Care and Use Committee. We have benefitted from comments on this work by G.F. Ball, J.L. Brown, S.C. Brown, E. Gwinner, J.C. Wingfield and two anonymous reviewers. Ryland Loos prepared the figure.

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