INVOLVEMENT OF A CIRCADIAN RHYTHM IN THE PHOTOPERIODIC OVARIAN RESPONSE OF THE YELLOW-THROATED SPARROW, *GYMNORHIS XANTHOCOLLIS*

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Summary

Groups of the photosensitive female yellow-throated sparrow were placed under various light–dark cycles, in which a fixed ultrashort photophase of 3 h was combined with dark phases of varying duration, resulting in cycles of 18–30 h. Simultaneously, two groups of birds, one in short days (8L/16D) and the other in long days (15L/9D), were kept as controls. Significant ovarian growth, observed at 30 and 60 days, was induced by 3 h ultrashort photophase only if it was introduced in the cycles of 18 h (3L/15D), 20 h (3L/17D), 26 h (3L/23D), 28 h (3L/25D) and 30 h (3L/27D) as well as under long days (15L/9D), whereas there was no response to the ultrashort photophase in cycles of 22 h (3L/19D) and 24 h (3L/21D) and in short days (8L/16D). It seems that there is an ovarian response to the ultrashort day cycles when a phase advance or delay of photosensitivity of the response system repeatedly produces coincidence of the external photophase (3 h) with the photosensitive phase of an endogenous circadian rhythm. The results are thus consistent with the Bünning hypothesis, which suggests the involvement of an endogenous circadian rhythm in photoperiodic time measurement.

Introduction

Since Rowan (1925) first discovered that day length was a primary environmental signal regulating the seasonal reproductive cycle in birds, a great deal of attention has been directed towards understanding the mechanism(s) by which day length is measured. There is considerable evidence that many birds utilize an endogenous circadian rhythm of sensitivity to daily light as a physiological basis of photoperiodism (Farner & Lewis, 1971; Follett, 1973; Farner, 1975; Turek, 1978; Turek *et al.* 1984). Such a concept was originally formulated by Bünning (1936) for plants and was experimentally demonstrated in a bird (*Carpodacus mexicanus*) by Hamner (1963). This hypothesis involves the operation of an external coincidence model, which predicts that a photoperiodic response is a result of a direct coincidence between the photosensitive phase of the entrained endogenous

circadian rhythm and the environmental photoperiod. This model attributes a dual role to light, i.e. entrainer and inducer (Pittendrigh & Minis, 1964).

One of the most powerful experimental paradigms that can be used to test for the involvement of the circadian system in photoperiodic time measurement is the use of ultrashort photoperiods in ahemeral (non 24 h) and hemeral (24 h) light–dark cycles. These cycles (T cycles) position the time of light at different phase points of the circadian system. Since a light–dark cycle involves control of both the phase and the period of the circadian system, if T is varied, the phase relationship of the circadian system to the light–dark cycle is altered as a function of the phase shift needed each day for the period of the endogenous rhythm to equal the period of the entraining light–dark cycle (Pittendrigh, 1981). Elliott (1976) has successfully used T cycles to map the circadian rhythm of sensitivity of the golden hamster's reproductive rhythm to 1 h light pulse.

Investigations involving the mechanism of photoperiodic time measurement in birds have mainly been confined to temperate-zone species and have used exclusively males. Very little attention has been paid to females (Follett & Sharp, 1969; Gwinner & Eriksson, 1977; Farner et al. 1966). To test the generality of the role circadian rhythmicity plays in avian photoperiodic time measurement, it is necessary to study more avian species and also the females of previously examined species (Turek & Campbell, 1979). The present experiment has not been extensively used with tropical/subtropical birds and, as far as we know, has never been carried out with females. Therefore, it has been designed to test the involvement of circadian rhythm(s) in photoperiodic time measurement in subtropical, seasonally breeding resident female yellow-throated sparrows, *Gymnorhis xanthocollis* (Burton), a photoperiodic species (Tewary & Dixit, 1986).

Materials and methods

Adult female yellow-throated sparrows were captured around Varanasi (latitude 25°18′N, longitude 83°01′E), India during December 1983 and housed in an outdoor aviary. They were first brought indoors to acclimatize them to laboratory conditions for 2 weeks. The acclimatized birds were then subjected to a pretreatment of short days (8L/16D) for 8 weeks to eliminate any residual photorefractoriness and render them sensitive to photostimulation. Monthly observations during the pretreatment period revealed that the birds had maintained their quiescent ovaries at a minimal mass (ovarian mass was about 4 mg). These photosensitive birds were used in the present study.

On 4th April 1984, the photosensitive yellow-throated sparrows were divided into seven groups and placed under various light–dark cycles, in which a fixed ultrashort photophase of 3 h was combined with dark phases of varying duration resulting in T cycles of 18–30 h. In addition, two control groups were maintained, one in short (8L/16D) and one in long (15L/9D) photoperiods (Table 1).

The light-proof boxes containing the birds were illuminated by 20 W fluorescent tubes, providing a light intensity of about 400 lx at perch level. The first light

Table 1. *Details of the seven ultrashort day cycles and two control cycles*

Group	Period of cycle (h)	Light schedule	Total number of cycles completed during experiment	Number of birds per group		
				0 days	30 days	60 days
G_1	24	8L/16D	60	6	5	5
G_2	24	15L/9D	60	6	5	5
G_{18}	18	3L/15D	80	6	5	5
G_{20}	20	3L/17D	72	6	5	5
G_{22}	22	3L/19D	65	7	7	7
G_{24}	24	3L/21D	60	7	6	6
G_{26}	26	3L/23D	55	6	5	5
G_{28}	28	3L/25D	51	6	5	5
G_{30}	30	3L/27D	48	7	6	6

period in all cycles was in phase with the pretreatment light schedule and commenced at 06.00 h. Photoperiodic chambers housing the birds were opened for feeding and laparotomies only during the light phase, which had been at a different time of the day for each group. Food and water were freshly and freely available to all the birds at all times and were resupplied when the lights were on. The frequency of such feeding was once per cycle. Observations of ovarian size were made on days 0, 30 and 60. The birds were laparotomized at each observation by surgically opening the abdominal wall between the last two ribs under anaesthesia. The ovarian mass of each bird was estimated visually by comparing the size of its ovary *in situ* with a reference set of fixed ovaries of known masses. The error inherent in this method may be ±20 %.

The data obtained from the present experiment were analysed using an analysis of variance. A two-factor mixed design (involving repeated measures) was used for this purpose. Supplemented Neumann–Keuls multiple range *t*-tests were employed at significance levels of 0·05 and 0·01 to ascertain the difference among mean values (Bruning & Kintz, 1977). The data from the birds that died during the experiment (see Table 1) were not included in the statistical analysis.

Results

The results are summarized in Fig. 1. There were significant variations in ovarian mass ($P < 0.001$) in relation to days ($F = 938.75$; df 2, 120) as well as cycles ($F = 425.03$; df 8, 120). The interactions of days and cycles were also significant ($F = 143.26$; df 16, 120; $P < 0.001$). A significant ovarian growth ($P < 0.01$) was evident in the birds of groups G_{18}, G_{20}, G_{26}, G_{28} and G_{30}, whereas the birds of groups G_{22} and G_{24} showed no response. Among the stimulated groups, the birds of groups G_{18}, G_{26}, G_{28} and G_{30} attained maximum ovarian mass ($P < 0.01$) on day 30. Ovarian regression was evident on day 60, although the average ovarian masses in all these groups were still significantly greater ($P < 0.01$) than their respective values on day 0. In contrast, an increase in ovarian mass above that
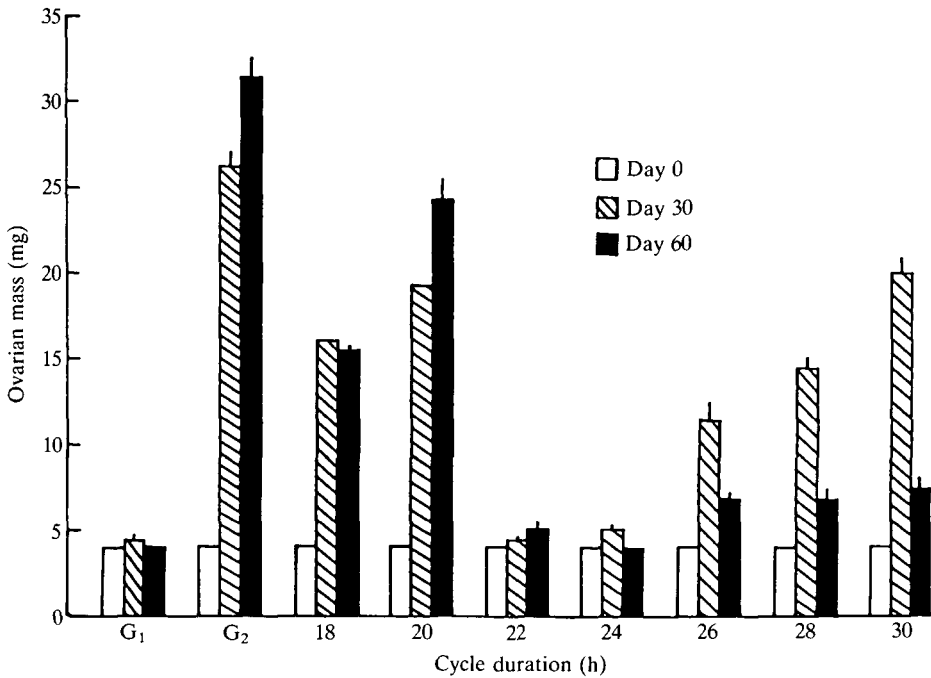
Fig. 1. Effect of ultrashort photoperiods on ovarian mass of yellow-throated sparrows. G_1 and G_2 represent the control groups held under short (8L/16D) and long (15L/9D) days, respectively. Standard error is represented by vertical bars if it extends beyond the limit of the point symbol.

attained by the birds of group G_{20} on day 30 ($P < 0.01$) was observed on day 60 ($P < 0.01$).

No ovarian growth was seen in the birds held under 8L/16D (G_1), whereas the control group exposed to 15L/9D (G_2) showed an increase in ovarian mass ($P < 0.01$) on day 30 and a further increase on day 60.

Discussion

It is interesting to note that the same photoperiod (3 h) available to all the experimental birds is effective only in combination with certain dark periods (15, 17, 23, 25 and 27 h), but it fails to induce ovarian growth when combined with other dark periods (19 and 21 h). These ultrashort day responses can best be interpreted by hypothesizing the operation of an endogenous circadian rhythm (Bünning, 1936; Pittendrigh & Minis, 1964). According to this view, photoperiodic induction and maintenance of gonadal growth depends on repeated (although not necessarily daily) coincidence of light with the photosensitive (= photoinducible) phase of an endogenous circadian rhythm. This has been reported in several other photoperiodic birds (Farner, 1975).

It is not clear how the ultrashort photophase of 3 h extends into the photoinduc

ible phase of an endogenous circadian rhythm that starts between 12 and 13 h after dawn in yellow-throated sparrow, as demonstrated by the night interruption experiment (Dixit, 1987). A possible explanation may be that if the phase advance or delay by the ultrashort day cycle, in a given duration, causes a coincidence (repeated, not necessarily daily) of the photoinducible phase with the external photophase, a photoperiodic response may occur. In the present investigation, an increase in ovarian mass was observed in the yellow-throated sparrow only in the cycles of 18, 20, 26, 28 and 30 h because the phase advance or delay by these cycles may ensure that the external photophase coincides repeatedly with the photo-inducible phase of the circadian rhythm. However, the increase in ovarian mass in the birds under 3L/17D on day 60, in contrast to the other groups, is surprising. A similar pattern of gonadal response has also been observed in male yellow-throated sparrows under these cycles (Tewary & Tripathi, 1986). The possibility of the onset of photorefractoriness in all the photostimulatory cycles, except for the birds in 3L/17D, is also possible. In contrast, no ovarian growth was noticed in birds under the cycles of 22 and 24 h. It is assumed that the phase advance or delay by these cycles may not be able to provide such coincidence and hence photoinduction of ovarian growth fails to occur. In the cycle of 24 h (3L/21D), the light period always falls in the nonphotoinducible phase, without any phase advance or delay of the cycle, precluding the possibility of any response. Thus, the results of the present experiment are in agreement with the results of resonance and night interruption experiments performed on yellow-throated sparrows (Tewary *et al.* 1984; Dixit, 1987).

Although these results demonstrate the involvement of endogenous circadian rhythm(s) during photoperiodic time measurement in this species and are consistent with the Bünning hypothesis, that is not the only hypothesis with which they are consistent. T cycles produced essentially negative results on photoinduced gonadal growth, raising queries about the importance of circadian rhythms in photoperiodic time measurement in Japanese quails. These cycles had 1–3 h of light and ranged in duration from 19 to 36 h and were ineffective in inducing testicular growth (Simpson & Follett, 1982). The results from various experiments (Simpson & Follett, 1982; Saiovici *et al.* 1987) suggest that photoperiodic time measurement relies on a clock that is triggered by dawn each day and shows only very weak circadian properties. In the light of these reports, further experiments involving the measurement of locomotor activity and the pattern of gonadotrophin secretion are required to confirm the mechanism of photoperiodic time measurement in the yellow-throated sparrow.

The ovary does not grow to full breeding condition in the yellow-throated sparrow under artificial photostimulation, although the testis reaches full spermatogenic levels under stimulatory light cycles (Tewary & Tripathi, 1986). Amongst the feral photoperiodic species, especially in passerines, it is known that, in general, only a partial development of the ovary can be induced by photoperiodic stimulation alone. The substantial reduction in ovarian response of photoperiodic birds is due to the failure of long daily photoperiods to induce vitellogenesis and

A. S. DIXIT AND P. D. TEWARY

the cumulative stages of follicular development. These changes, which occur in a relatively short time, clearly require essential supplementary information from the environment, such as presence of an active mate, of nesting material or of a nest site (Farner & Lewis, 1971; Farner & Follett, 1979; Wingfield, 1983), and possibly the cessation of inhibitory environmental information (Yokoyama & Farner, 1976). Ovarian growth and maturation in photoperiodic species is biphasic; the initial slow growth phase may begin several weeks to several months before the prospective breeding season, followed by a rapid growth phase, during which yolk is deposited and which occupies the last 4–11 days before ovulation (Farner & Lewis, 1971). Thus, it seems that the circadian photoperiod measurement mechanism is only relevant to the first phase. Another, possibly non-photoperiodic, mechanism takes over for the final phase of ovarian development, suggesting that there is a sex difference in the mechanisms of gonadal recrudescence, since males exhibit full testicular development with photoperiodic stimulation only.

The literature on avian photoperiodism is not extensive regarding the ultrashort photoperiodic gonadal responses in ahemeral and 24 h cycles. Only a few species have been investigated under these light regimes. The gonadal response of yellow-throated sparrows under a 3L/21D light regime is similar to that of *Zonotrichia leucophrys gambelii* (Farner *et al.* 1977), *Emberiza bruniceps* (Tewary *et al.* 1982), *Emberiza melanocephala* (Tewary & Kumar, 1983), but is inconsistent with that of *Lonchura punctulata* (Chandola & Thapliyal, 1977) and *Sturnus vulgaris* (Schwab, 1971), in which this cycle is gonadostimulatory. Moreover, gonadal response under 3L/23D and 3L/25D resembles that of the temperate-zone population of *Passer domesticus* (Farner *et al.* 1973, 1977). The gonadal response of the yellow-throated sparrow under 3L/23D differs from that of *Zonotrichia leucophrys gambelii* (Farner *et al.* 1977) and *Emberiza bruniceps* (Tewary *et al.* 1982) but is similar to that of *Lonchura punctulata* (Chandola *et al.* 1975; Thapliyal *et al.* 1975) and *Carpodacus mexicanus* (Hamner & Enright, 1967).

Thus, it may be concluded from the present results that the ovarian responses of the yellow-throated sparrow under T cycles are consistent with the Bünning hypothesis. However, the confirmation of such a mechanism requires further investigations involving recordings of motor activity.

References

BRUNING, J. L. & KINTZ, B. L. (1977). *Computational Handbook of Statistics* (2nd edn). Illinois: Glenview.

BÜNNING, E. (1936). Die endonome Tagesrhythmik als Grundlage der photoperiodische Reaktion. *Ber. dt. bot. Ges.* **54**, 590–607.

CHANDOLA, A., PAVNASKAR, J. & THAPLIYAL, J. P. (1975). Scoto/photoperiodic responses of a subtropical finch (spotted munia) in relation to seasonal breeding cycle. *J. interdiscipl. Cycle Res.* **6**, 189–202.

CHANDOLA, A. & THAPLIYAL, J. P. (1977). Photoperiodic responses of Indian weaver birds *Proc. Inst. Symp. avian Endocri. Calcutta* 60–72.

DIXIT, A. S. (1987). Photoperiodicity in some female birds. PhD dissertation, Banaras Hindu University, Varanasi, India.

ELLIOTT, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. *Fedn Proc. Fedn Am. Socs exp. Biol.* **35**, 2339–2346.

FARNER, D. S. (1975). Photoperiodic controls in the secretion of gonadotropins in birds. *Am. Zool.* **15**, 117–135.

FARNER, D. S., DONHAM, R. S., LEWIS, R. A., MATTOCKS, P. W., JR, DARDEN, T. R. & SMITH, J. P. (1977). The circadian component in the photoperiodic mechanism of the house sparrow, *Passer domesticus. Physiol. Zool.* **50**, 247–268.

FARNER, D. S. & FOLLETT, B. K. (1979). Reproductive periodicity in birds. In *Hormones and Evolution* (ed. E. J. W. Barrington), pp. 829–872. London: Academic Press.

FARNER, D. S., FOLLETT, B. K., KING, J. R. & MORTON, M. L. (1966). A quantitative examination of ovarian growth in the white-crowned sparrow. *Biol. Bull. mar. Biol. Lab., Woods Hole* **130**, 67–75.

FARNER, D. S. & LEWIS, R. A. (1971). Photoperiodism and reproductive cycles in birds. In *Photophysiology*, vol. 6 (ed. A. C. Giese), pp. 325–370. New York, London: Academic Press.

FARNER, D. S., LEWIS, R. A. & DARDEN, T. (1973). Photostimulation of gonads in birds: the Bünning hypothesis and responses to very short photoperiods. In *UNESCO Int. Congr. The Sun in the Service of Mankind*, Paris **13**, 1–10.

FOLLETT, B. K. (1973). Circadian rhythms and photoperiodic time measurement in birds. *J. Reprod. Fertil.* **19**, 5–18.

FOLLETT, B. K. & SHARP, P. J. (1969). Circadian rhythmicity in photoperiodically induced gonadotropin release and gonadal growth in the quail. *Nature, Lond.* **223**, 968–971.

GWINNER, E. G. & ERIKSSON, L. O. (1977). Circadiane Rhythmik und photoperiodische Zeitmessung beim Star (*Sturnus vulgaris*). *J. Ornithol.* **118**, 60–67.

HAMNER, W. M. (1963). Diurnal rhythm and photoperiodism in testicular recrudescence of the house finch. *Science* **142**, 1294–1295.

HAMNER, W. M. & ENRIGHT, J. T. (1967). Relationship between photoperiodism and circadian rhythms of activity in the house finch. *J. exp. Biol.* **46**, 43–61.

PITTENDRIGH, C. S. (1981). Circadian systems: General perspective. In *Handbook of Behavioural Neurobiology*, vol. 4 (ed. J. Aschoff), pp. 57–80. New York: Plenum Press.

PITTENDRIGH, C. S. & MINIS, D. H. (1964). The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Am. Nat.* **98**, 261–294.

ROWAN, W. (1925). Relation of light to bird migration and developmental changes. *Nature, Lond.* **115**, 494–495.

SAIOVICI, M. S., NICHOLLS, T. J. & FOLLETT, B. K. (1987). Rapid photoperiodic response in Japanese quail: Is daylength measurement based upon a circadian system? *J. biol. Rhy.* **2**, 139–152.

SCHWAB, R. G. (1971). Circannian testicular periodicity in the European starling in the absence of photoperiodic change. In *Biochronometry* (ed. M. Menaker), Nat. Acad. Sci., pp. 428–447, Washington.

SIMPSON, S. M. & FOLLETT, B. K. (1982). Formal properties of the circadian system underlying photoperiodic time measurement in Japanese quail. *J. comp. Physiol.* **145**, 381–390.

TEWARY, P. D. & DIXIT, A. S. (1986). Photoperiodic regulation of reproduction in subtropical female yellow-throated sparrow (*Gymnorhis xanthocollis*). *Condor* **88**, 70–73.

TEWARY, P. D., DIXIT, A. S. & KUMAR, V. (1984). Circadian rhythmicity and the initiation of reproductive functions in female passerines. *Physiol. Zool.* **57**, 563–566.

TEWARY, P. D. & KUMAR, V. (1983). Control of testis function in blackheaded bunting, *Emberiza melanocephala. Curr. Sci.* **53**, 113–114.

TEWARY, P. D., PRASAD, B. N. & KUMAR, V. (1982). Circadian basis of photoperiodically induced testicular growth in redheaded bunting, *Emberiza bruniceps. Anim. Reprod. Sci.* **4**, 245–249.

TEWARY, P. D. & TRIPATHI, P. M. (1986). Response to ultra-short photoperiods by the subtropical yellow-throated sparrow, *Gymnorhis xanthocollis. Anim. Reprod. Sci.* **10**, 237–243.

THAPLIYAL, J. P., CHANDOLA, A. & PAVNASKAR, J. (1975). Effects of continuous light and very

short photoperiods on the gonadal function of a subtropical finch, spotted munia, *Lonchura punctulata. J. Endocr.* **65**, 141–142.

TUREK, F. W. (1978). Diurnal rhythms and the seasonal reproductive cycle in birds. In *Environmental Endocrinology* (ed. I. Assenmacher & D. S. Farner), pp. 144–152. New York: Springer-Verlag.

TUREK, F. W. & CAMPBELL, C. S. (1979). Photoperiodic regulation of neuroendocrine–gonadal activity. *Biol. Reprod.* **20**, 32–50.

TUREK, F. W., SWANN, J. & EARNEST, D. J. (1984). Role of the circadian system in reproductive phenomena. *Rec. Prog. Horm. Res.* **40**, 143–183.

WINGFIELD, J. C. (1983). Environmental and endocrine control of avian reproduction: An ecological approach. In *Avian Endocrinology* (ed. S. Mikami, K. Homma & M. Wada), pp. 263–288. Berlin, Heidelberg, New York: Springer-Verlag.

YOKOYAMA, K. & FARNER, D. S. (1976). Photoperiodic responses in bilaterally enucleated female white-crowned sparrows *Zonotrichia leucophrys gambelii. Gen. comp. Endocr.* **30**, 528–533.