24. Yeoman, M. M., Miedzybrodzka, M. B., Lindsey, K., McLauchlan, W. R., in *Plant Cell Cultures. Results and Perspectives* (eds Sala, F., Parisi, B., Cella, R. and Ciferri, O.), Elsevier, Amsterdam, 1980, pp. 327–343.
25. Lindsey, K., Yeoman, M. W., *Cell Culture and Somatic Cell Genetics of Plants. A Comprehensive Treatise* (ed. Vasil, I.), Academic Press, New York, 1985, pp. 61–101.
26. Lindsey, K. and Yeoman, M. M., in *Plant Biotechnology – Society for Experimental Biotechnology Seminar Series 18* (eds Smith, H. and Mantell, S. M.), Cambridge University Press, Cambridge, 1983, pp. 39–66.
27. Chowdhury, A. R. and Chaturvedi, H. C., *Curr. Sci.*, 1979, **49**, 237–238.
28. Payne, G., in *Plant Cell and Tissue Culture in Liquid Systems* (eds Payne, G., Bringi, V., Prince, C. and Shuler, M.), Hanser Publishers, Munich, 1991, pp. 225–280.

Received 29 March 1997; revised accepted 26 December 1997

Photoperiodic regulation of reproduction in migratory bunting, *Emberiza melanocephala*: An evidence for external coincidence model

Manoj Kumar Mishra and P. D. Tewary

Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Photosensitive female blackheaded buntings (*Emberiza melanocephala*) were exposed to various night-interruption cycles for 42 days. These light–dark cycles consisted of a basic photophase of 6 h and 1 h photointerruption of the 18 h dark phase in 24 h cycle at different points. A control group was also placed under short photoperiod (8L : 16D). Ovarian growth and increased plasma estradiol level were evident in night interruption cycle in which photo-interruption of dark photophase was made 13 h and 15 h after the onset of basic photophase, i.e. 6L : 7D : 1L : 10D and 6L : 9D : 1L : 8D cycles. The present results are consistent with external coincidence model (Bünning hypothesis), suggesting the involvement of an endogenous circadian rhythm in photoperiodic time measurement in bunting.

SEVERAL avian species of temperate, subtropical and tropical zones have been reported to display their reproductive, physiological, morphological and behavioural activities in response to environmental cues in which the role of photoperiod is of great importance[1–5]. Endogenous circadian rhythm and its involvement in control of reproduction in avian as well as in mammalian species with regard to photoperiod have been well defined[2,6–9]. Many powerful methods have been used to test the circadian rhythm of sensitivity to light, of which night-interruption experimental protocol is worth mentioning. It involves the use of repetitive light–dark cycles consisting of a main photophase of non-stimulatory duration combined with dark phase in 24 h cycle which is interrupted by 1 h short pulse of light. Using this protocol, various experiments have been carried out on avian species, particularly on males paying less attention towards females[10–13]. Keeping in view the scanty information about female avian species, we sought to explore the role of photoinducible phase and involvement of the circadian rhythm in photoperiodic time measurement in female blackheaded bunting.

The blackheaded bunting is a migratory finch which visits the Indian subcontinent during winter season (September/October) and returns to its breeding ground when summer approaches (March : April)[14]. Buntings were captured locally near Varanasi (25°18′N, 83°01′E) in January 1995 when they were available in plenty. They were brought to an outdoor aviary, acclimatized for 15 days and then placed in a short photoperiodic (8L : 16D) environment for 60 days to make them photosensitive. Birds were laparotomized periodically to ensure that they maintained regressed ovaries (4–5 mg). These photosensitive birds were divided into six groups (five experimental and one control) and kept in wirenet cages (50 × 30 × 25 cm). The control group was placed under short photoperiod (8L : 16D) while the experimental groups were held under various night interruption cycles such as 6L : 3D : 1L : 14D (9 h), 6L : 5D : 1L : 12D (11 h), 6L : 7D : 1L : 10D (13 h), 6L : 9D : 1L : 8D (15 h) and 6L : 11D : 1L : 6D (17 h) for 42 days (Table 1). The cages containing birds (4 birds/cage) were placed under different photoperiodic chambers (as scheduled above), illuminated with 20 W fluorescent tubes having light intensity of 400 lux at perch level. The food and water were supplied *ad libitum* only during light phase of cycles which commenced in each cage at 0600 h. The birds were laparotomized before the beginning and at the end of experiments and ovarian growth was assessed by comparing the size of ovaries *in situ*, with standard sets of ovaries of known weights. The error inherent in this method is about 20% (ref. 15). In the beginning and at the end, blood was collected in heparinized capillary tubes, centrifuged at 4°C, plasma separated and kept at −20°C till assayed. The circulating plasma estradiol concentration was measured by radioimmunoassay using estradiol Direct Radioimmunoassay kit (Biotexc Laboratories Inc., 6023, Southloop East Huston, Tx 77033). The kit was highly specific for estradiol. The cross reactivity for 17β-estradiol was 100%. Data were analysed using one-way analysis of variance (ANOVA) supplemented with Neuman–Keul's multiple range *t*-test.

Table 1. Effect of various night interruption photoperiodic schedules on ovarian growth and plasma estradiol level of female blackheaded bunting. The 6L : 7D : 1L : 10D and 6L : 9D : 1L : 8D showed significant increase ($P < 0.001$) in ovarian weight and plasma estradiol level while rest group showed no response. 6L : 11D : 1L : 6D exhibited slight increase in ovarian weight and plasma estradiol level which was statistically insignificant. In the beginning of the experiment all birds showed a regressed ovarian state (about 5 mg) and undetectable level of plasma estradiol (< 10 pg/ml)

Photoperiodic schedule	Time of interruption of 1 h dark phase in each cycle	Ovarian weight (mg)	Plasma estradiol (pg/ml)
8L : 16D	Control	4.75 ± 0.14	< 10
6L : 3D : 1L : 14D	9	5.95 ± 0.21	< 10
6L : 5D : 1L : 12D	11	4.57 ± 0.14	< 10
6L : 7D : 1L : 10D	13	18.92 ± 0.60*	280.0 ± 17.32*
6L : 9D : 1L : 8D	15	16.35 ± 0.75*	200.0 ± 34.64*
6L : 11D : 1L : 6D	17	6.55 ± 0.90	21.6 ± 4.16

*$P < 0.01$, ± denotes SEm.

Table 1 shows the significant variation in ovarian gowth (F-value 133.335, df 18, 5; $P < 0.001$) and circulating plasma estradiol level (F-value 176.96, df 12, 5; $P < 0.001$) during various night interruption light dark schedules. The photoperiodic groups of 6L : 7D : 1L : 10D (13 h) and 6L : 9L : 1L : 8D (15 h) showed significant increase ($P < 0.01$) in ovarian growth and plasma estradiol level in comparison with control and other groups. Although the 6L : 11D : 1L : 6D (17 h) group showed a little increase in ovarian growth, statistically it was not significant. Circulating plasma estradiol concentration and ovarian growth of 6L : 7D : 1L : 10D (13 h) group differ significantly ($P < 0.01$) from those of 6L : 9D : 1L : 10D (15 h) group. The present result clearly suggests that the photostimulation in female blackheaded bunting occurs under a basically noninductive short day length (6 h) when an additional very short light pulse (1 h) was introduced during long dark phase. This supports the existence of an endogenous circadian component which plays an important role in timing and triggering the induction of a positive photoperiodic response in bunting. In all groups only 7 h light pulse was given (6 h basic photophase + 1 h photointerruption at different points) which is far less than the threshold photoperiodicity (12–13 h) of the female bunting (unpublished data). This clearly suggests the importance of position of light during the circadian rhythm of photosensitivity of bird which contains a photoinducible phase which when illuminated caused positive response. Light : dark regimes of this nature are called skeleton photoperiods and they successfully mimic complete photoperiods. Thus, the present finding supports the Bünning hypothesis[16] and their interpretations are in total agreement with external coincidence model. Photoperiodically controlled avian gonadal response may be explained as the result of coincidence between the photoinducible phase of an entrained circadian component within the bird and environmental photoperiod. Photostimulation fails to occur when an appropriate relation between the photoinducible phase and light is not achieved[3,10,12,13,17–19]. Photostimulation causes ovarian growth and increase in plasma estradiol concentration[20–23] while short day length causes decrease in ovarian growth and plasma estradiol concentration[24]. The undetectability of plasma estradiol in photosensitive buntings and in some experimental groups shows similarity with white-browed sparrow weaver in which high plasma estrogen was detectable only prior to ovulation and most samples showed undetectable levels of estrogen[24]. To sum up, it is clear that the female blackheaded bunting possesses an endogenous circadian component and a distinct photoinducible phase (12–13 h) which when illuminated leads to photoperiodic response and is an evidence for the external coincidence model.

1. Farner, D. S. and Follett, B. K., *J. Anim. Sci.*, 1966, **25**, 90–118.
2. Tewary, P. D. and Tripathi, B. K., *J. Exp. Zool.*, 1983, **226**, 269–272.
3. Ravikumar, G., Senthilkumaran, B., Tewary, P. D. and Goel, A. K., *J. Biol. Rhythm*, 1995, **10**, 319–323.
4. Wingfield, J. C., Hahn, T. P., Levin, R. and Honey, P., *J. Exp. Zool.*, 1992, **261**, 214–231.
5. Wingfield, J. C., Hahn, T. P. and Doak, D., in *Avian Endocrinology* (ed. Sharp, P. J.), Journal of Endocrinology Ltd, Bristol, 1993.
6. Kumar, V. and Tewary, P. D., *IBIS*, 1983, **125**, 305–312.
7. Farner, D. S., *Annu. Rev. Physiol.*, 1985, **47**, 65–82.
8. Malpaux, B., Robinson, J. E., Wayne, N. L. and Karsh, F. J., *J. Endocrinol.*, 1989, **122**, 267–278.
9. Sweeny, T., Donoran, A., Roche, J. F. and Callaghan, D. O., *J. Reprod. Fertil.*, 1997, **109**, 121–127.
10. Dixit, A. S. and Tewary, P. D., *Acta Physiol. Hungarica*, 1989, **72**, 457–462.
11. Tewary, P. D. and Kumar, V., *Indian J. Exp. Biol.*, 1981, **19**, 77–79.
12. Follett, B. K., Kumar, V. and Juss, T., *J. Comp. Physiol.*, 1992, **171**, 533–540.
13. Kumar, V. and Kumar, B. S., *J. Exp. Zool.*, 1995, **273**, 297–302.
14. Ali, S. and Ripley, S. D., *Handbook of Birds of India and Pakistan*, Compact edition, Oxford University Press, Delhi, 1983, vol. 10.
15. Tewary, P. D., Tripathi, B. K. and Kumar, V., *Gen. Com. Endocrinol.*, 1983, **52**, 315–318.
16. Bünning, E., *Ber. Dtsch. Bot. Ges.*, 1936, **54**, 590–607.
17. Follett, B. K., *J. Reprod. Fertil.*, 1973, **19**, 5–18.
18. Follett, B. K., *Gen. Com. Endocrinol.*, 1981, **45**, 306–316.

19. Farner, D. S., *Am. Zool.*, 1975, **15**, 117–135.
20. Reinert, B. D. and Wilson, F. E., *Gen. Com. Endocrinol.*, 1996, **103**, 60–70.
21. Boswell, T., Hall, M. R. and Goldsmith, A. R., *J. Zool. London*, 1993, **231**, 627–644.
22. Schwabl, H., Schwabl-Benzinger, I., Goldsmith, A. R. and Farner, D. S., *Gen. Comp. Endocrinol.*, 1988, **71**, 398–405.
23. Brain, P. C., Onagbesan, O. M., Peddie, M. J. and Tayler, T. G., *Gen. Com. Endocrinol.*, 1988, **69**, 174–180.
24. Wingfield, J. C., Hegner, R. E. and Levis, D. M., *J. Zool. London*, 1991, **225**, 23–58.

ACKNOWLEDGEMENT. This work was supported by a grant from the Council of Scientific and Industrial Research to P.D.T.

Received 17 October 1997; revised accepted 17 December 1997.

Incidence of parental care in the cockroach *Thorax porcellana* (Saravas) (Blaberidae: Blattaria)

S. Bhoopathy

Department of Zoology, Voorhees College, Vellore 632 001, India

In the ovoviviparous cockroach *Thorax porcellana*, the first two nymphal instars live under the dome-shaped elytra of their mother. Young ones are completely dependent upon the mother during these stages. Modified mandibles of the nymphs help them in adhering to the surface of the mother and to derive nourishment from the mother. Observations are based on the studies made on the living specimens.

PARENTAL care in cockroaches is mostly limited to the strategies of the female for concealing their oothecae. The viviparous cockroaches keep their oothecae within their bodies until the eggs hatch and thus protect them from desiccation and attack from predators and parasites[1,2]. Contributions to the studies on parental care in such species include those of Shelford[3,4] on *Pseudophoraspis nebulosa* and *Phlebonotus pallens*, Pruthi[5] on *Phlebonotus pallens*, Hanitsch[6] on *Perisphaerus glomeriformis* and *Ellipsidion variegatum*, Karny[7] on phorasphinae, Gurney[8] on *Perisphaerus* sp., Sein[9], Pesosa and Correa[10] and Wolcott[11] on *Leucophaea maderae*. Breed *et al.*[12] showed that females of *Blattella germanica* were more aggressive.

In the social cockroach, *Cryptocercus punctulatus* which is monogamous and morphologically, ethologically and physiologically related to lower termites; the nymphs feed by trophallaxis on adult proctodaeal materials[13]. Nalepa[14] studied the colony composition of this wood roach in detail. Liechti and Bell[15] noticed that the nymphs of *Byrzeteria fumigata* aggregate under the female until their cuticle hardens.

Bhoopathy[16–18] studied oothecal structural modifications with reference to ovoviviparous nature of *T. porcellana*, their microhabitat preferences and their mating behaviour.

Roth[19] made some observations on the arched tegmina in pinned museum specimens of the genus *Thorax* (*Saussure*) and suggested that they may function in a maternal care role. The present study brings to light the unknown details of the mother–offspring relationship in *Thorax porcellana* (Saravas) on the basis of observations made on live specimens.

For the collection of *T. porcellana*, the forest area abounding the Indian Institute of Technology, Guindy, Chennai was chosen. This species of cockroach occupies a unique microhabitat, occurring amidst clumps of dried leaves held between the intertwining branches of dense shrubs such as *Prosopis spicigera*, *Gymnosporia montana* and *Flacourtia* sp. (Figure 1 *a*). The site was natural with least human interference. This is a univoltine species, having a single generation per year. The peculiar microhabitat of this species and their solitary habit prevents building up of dense populations. Adults were found only in August, September and October, while the nymphal population was at its peak in April and May. The nymphs and the adults of this species do not expose themselves to temperature extremes.

Beating the shrubs with a wide-mouthed net placed underneath is the method used for their collection. In *T. porcellana*, which is ovoviviparous, the ootheca becomes retracted into the genital pouch of the mother. To determine the period of incubation of eggs, adult males and females were kept in rearing jars and soon after mating, the females were isolated and kept in a separate jar. They were fed on a natural diet of dried leaves of the above-mentioned shrubs and water.

They were observed daily for recording the emergence of the first instar nymphs which crawl over the mother and hide beneath the elytra of the mother; the nymphs are, however, discernible only when the female is closely viewed at her abdominal pleural region, where a narrow gap is let out by the elytra between them and abdominal dorsum exposing part of the nymphs. Thus, the incubation period could be determined with reasonable precision.

The nymphs were collected from the mother for studying the mandibles and head. The head and mandibles of nymphs and adults after clearing in KOH, dehydrated with alcohol were cleared in clove oil and mounted on Canada Balsam on slides. Photographs were taken for further study.

In *T. porcellana*, an ootheca containing 34–40 eggs was formed, however, due to the ovoviviparous habit of this species, the ootheca which was smooth and flexible, after an initial extrusion, retracted into the genital chamber and was protected in the uterus where