the average pappus part numbers in individual heads of C30 × C93 hybrids (expected phenotype 7.5) on the date of the harvest. The same effect is seen, if on a much smaller scale, in the parental selfs obtained in these crosses (fig. 2). Obviously it is greatly magnified by the partial breakdown of numerical canalization through the addition of the 5-gene in the hybrids.

Taking into account phenotypic instability, which can be monitored by comparing the pappus part numbers in early and late capitula, the results of these crosses can be interpreted rather reliably (table). All the tested strains of *M. pygmaea* contain either one or two 10-determining pappus part genes. Additional duplication events creating four pappus part genes in the genome, as found in some strains of *M. bigelovii*, have not been detected in *M. pygmaea*. However, fitting the gene numbers found here with our scheme of the evolution of *M. pygmaea* based on enzyme gene mutations[6] requires us to assume two independent duplications of the original pappus part gene (fig. 3). One of these has led to two 10-genes in one of the coastal strains (C99), while the other must have occurred far inland, south of Santiago. This result immediately suggests further tests of our evolutionary hypothesis: All strains of *M. pygmaea* should have one 10-gene allelic to the single 5-gene from C93 (to support the notion of a single common ancestral gene). The second 10-gene in strains A92, C37 and C97 should map to another chromosome (as it does in C37) but in all three to the same (to support the common origin of the second 10-gene in these inland strains), while a different location of the second 10-gene in strain C99 would strongly support the origin of this gene by an independent duplication event.

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1 Chambers, K. L., Contr. Dudley Herb. *4* (1955) 207.
2 Bachmann, K., and Chambers, K. L., Pl. Syst. Evol. *129* (1978) 119.
3 Bachmann, K., Chambers, K. L., and Price, H. J. Pl. Syst. Evol. *138* (1981) 235; Bachmann, K., Chambers, K. L., Price, H. J., and König, A., Pl. Syst. Evol. *141* (1982) 123; Bachmann, K., Chambers, K. L., and Price, H. J., Pl. Syst. Evol. *145* (1984) 243.
4 Bachmann, K., Sonderb. Naturwiss. Ver. Hamburg *7* (1983) 65.
5 Carlquist, S., Am. Sci. *69* (1981) 509.
6 Bachmann, K., Chambers, K. L., Grau, J., and Price, H. J., Beitr. Biol. Pflanzen (1985); in press.
7 Bachmann, K., Chambers, K. L., Price, H. J., and König, A., Beitr. Biol. Pflanzen *57* (1983) 167.
8 Mauthe, S., Bachmann, K., Chambers, K. L., and Price, H. J., Biol. Zbl. *104* (1985) 1.
9 Bachmann, K., and Chambers, K. L., Experientia *37* (1981) 29.

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Circadian periodicity in termination of photorefractoriness in the yellow-throated sparrow[1]

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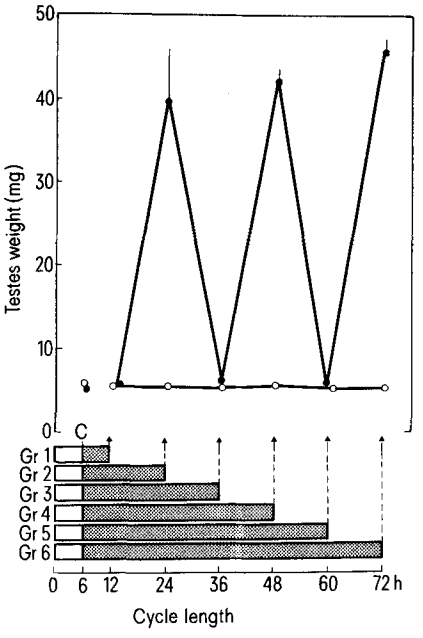
Summary. Photorefractory male yellow-throated sparrows *(Gymnorhis xanthocollis)* were subjected to six resonance light cycles, consisting of 6 h basic photophase coupled with scotophases of longer durations (in cycles of 12 (6L:6D) h, 24 (6L:18D) h, 36 (6L:30D) h, 48 (6L:42D) h, 60 (6L:54D) h and 72 (6L:66D) h). Termination of photorefractoriness was evident in cycles of 24, 48 and 72 h, but not in 12, 36 and 60 h. The results are consistent with the 'external-coincidence' model (Bünning hypothesis) and suggest that endogenous circadian rhythmicity is involved in the termination of photorefractoriness.

Key words. Photorefractory; yellow-throated sparrow; resonance; photophase; scotophase; Bünning hypothesis; circadian rhythmicity.

Termination of the refractory period in some avian species involves a circadian component during the photoperiodic time measurement[3-8]. Investigations have, however, been limited to temperate species or to those palearctic ones which visit the Indian subcontinent or Southeast Asia. The present study was planned, therefore, to test the involvement of endogenous circadian rhythm (ECR) of photosensitivity during the termination of refractoriness in a subtropical yellow-throated sparrow *(Gymnorhis xanthocollis)*. The experimental design was similar to that developed first by Nanda and Hamner[9], now widely used as a tool for exploring circadian mechanisms. The sparrow is a resident and marked local migrant[10]. It has a seasonal reproductive cycle with a post-reproductive refractory period that needs about 8-weeks of short day (8L:16D) treatement for its dissipation (Tewary and Tripathi, unpublished data).

Materials and methods. Birds (n = 70) captured during January 1983 around Varanasi (India, lat. 25°18'N, long. 83°01'E.) were acclimatized in an outdoor aviary and moved into a short-day environment for 2 months. Such photosensitive birds were subjected to long-day conditions (15L:9D) for 120 days to make them photorefractory. During this period they were periodically examined. Completely regressed gonads (CTW = about 5 mg), followed by maximum gonadal growth (CTW = about 120 mg) indicated their photorefractory state. On 29 July 1983 such refractory birds in six groups (n = 6 each), and a control, were challenged with different programmed resonance light/dark cycles consisting of 12 (6L:6D) h, 24 (6L:18D) h, 36 (6L:30D) h, 48 (6L:42D) h, 60 (6L:54D) h and 72 (6L:66D) h for 60 days. Birds were reexamined and transferred to 15L:9D for the next 30 days to determine the dissipation of photorefractoriness. The control group was maintained under long day conditions (15L:9D) throughout experimentation. Light control was automatic (VEB Zeitschaltelektronik, Frauenstein, GDR) and also manual. Food and water were available ad libitum and were changed during the light period. All birds were lit by 20-W fluorescent tube rods, yielding an intensity of about 400 lux at cage level; the first experimental photophase was in phase with pretreatment and commenced at 06.00 h. The weight of the testes was evaluated in situ by an exploratory unilateral laparotomy as combined testicular weight (CTW), by comparison with fixed gonads of known weight. The error inherent in this method was less than 15%. Data were analyzed by Student's t-test.

Results and discussion. The data presented in the figure demonstrate that testicular recrudescence ($p < 0.001$) was evident after transfer to 15L:9D in yellow-throated sparrows which had previously been maintained under cycles of 24, 48 and 72 h (perceived as short days), but not in the control group or in those maintained under cycles of 12, 36 or 60 h (perceived as long days), despite the fact that all cycles had received only 6 h basic photophase. No significant testicular growth was evident in either the initial, finally regressed or the stimulated groups.



Testicular responses of yellow-throated sparrows under resonance light-dark cycles after 30 days of transfer to long day (15L:9D). Open bar is 6 h basic photophase. Stippled bars represent longer duration of scotophase. C is control group. Open and solid circles represent the initial and final testes weights. Gr represents groups. Vertical bars represent the statistical error if it exceeds the limits of the point symbol. Seven birds, one from each group, died at the end of the experiment. Data from dead birds were excluded in our analysis.

The results are in agreement with those obtained for the white-crowned sparrow and golden-crowned sparrow[5], Redheaded Bunting[7] and Blackheaded Bunting[8] (although in the latter, 72 h cycle was not investigated). Further, in the white-crowned sparrow a 16 (8L:8D) h cycle dissipated the refractory period, although it maintained it in the golden-crowned sparrow[5], but the reason for that is unknown. However, our results suggest that an endogenous circadian rhythm is involved in photoperiodic time measurement during the termination of the refractory period in *G. xanthocollis*. Thus, the results can be interpreted on the basis of an 'external-coincidence' model (Bünning hypothesis), which suggests that the termination of the refractory period depends on the non-coincidence of light (daily or otherwise) with an endogenous circadian rhythm of photosensitivity[11,12].

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2 To whom reprint requests should be addressed.
3 Hamner, W.M., Ecology *49* (1968) 211.
4 Murton, R.K., Lofts, B., and Westwood, N.J., Gen. comp. Endocr. *14* (1970) 107.
5 Turek, F.W., Science *178* (1972) 1112.
6 Sansum, E.L., and King, J.R., J. comp. Physiol. *98* (1975) 183.
7 Prasad, B.N., and Tewary, P.D., Physiol. Zool. *56* (1983) 64.
8 Tewary, P.D., and Kumar, V., Condor *86* (1984) 27.
9 Nanda, K.R., and Hamner, K.C., Bot. Gaz. *120* (1958) 14.
10 Whistler, H., in: Popular handbook of Indian birds, p. 224. Gurney and Jackson, London, Edinburgh 1928.
11 Farner, D.S., Am. Zool. *15*, suppl. (1975) 117.
12 Turek, F.W., in: Diurnal rhythms and the seasonal reproductive cycle in birds, p. 144. Eds I. Assenmacher and D.S. Farner. Environmental endocrinology, Springer, Berlin, Heidelberg, New York 1978.

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Anaerobic microbial conversion of three hydroxycinnamic acids

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Summary. Caffeic, ferulic and synapic acid are anaerobically degraded by a microbial consortium via the reduction of the double bond of the side chain, the replacement of the nuclear hydroxyl group in position 4 by hydrogen, the demolition of the side chain by the loss of a C_1 unit.
Key words. Anaerobic metabolism; phenylpropanoid catabolism; methanogenic consortium; metabolic sequence; phenolic acid degradation.

Phenolic acids frequently occur in plants as hydroxycinnamic acids ester-linked to polysaccarides and large amounts of simple phenolic acids, such as ferulic (**1**), p-coumaric (**2**) and syringic acids (**3**), are released during the aerobic catabolism of lignin[1]. The aerobic catabolism of these aromatic substances has been largely studied[2,3]; on the contrary the anaerobic degradation of the simplest aromatic lignin derivatives has not received much attention. Healy et al.[4] observed that ferulic acid (**1**) is biodegradable to methane and carbon dioxide under strict anaerobic conditions by a methanogenic consortium of bacteria through initial ring reduction followed by ring cleavage. Kaiser and Hanselmann[5] reported that syringic acid (**3**) is completely degraded to methane and carbon dioxide via the intermediate formation of gallic acid (**4**) by two demethylation steps.

Our attention was focused on the reactions involved in the early steps of the anaerobic pathway of different hydroxycinnamic acids by the bacterial community from an anaerobic sewage sludge.

Anaerobic enrichment cultures of this bacterial community were obtained in prereduced mineral medium FW[6] added of 2‰ (w/v) trypticase, 2‰ (w/v) peptone and 0.5‰ (w/v) caffeic acid (**5**), dispensed into rubber sealed bottles. Anaerobic conditions were assured by flushing the bottles with a mixture of hydrogen and carbon dioxide (80:20). Resazzurrin was added at a final concentration of 0.001% as a redox indicator. Unter these conditions the disappearance of the UV absorption spectrum of caffeic acid (**5**) was observed after 20 days of incubation.

When prereduced mineral medium FW, in which caffeic acid (**5**) was the only carbon and energy source, was inoculated with the consortium caffeic acid (**5**) (scheme 1) was transformed after a 5-day incubation into 3,4-dihydroxyphenylpropionic acid (**6**); 3,4-dihydroxyphenylacetic acid (**7**) and 3,4-dihydroxyphenylpropionic acid (**8**). After a 30-day incubation compounds (**6**) and (**8**) were still present, together with 3-hydroxyphenylacetic acid (**9**).

All these compounds were detected by GLC-MS after diazomethane treatment of the crude extract at pH = 2 and were identified by comparison with authentical specimens.

Since the appearance of some metabolites seemed to depend from the incubation time, a study related to the sequence of these