concentrations of GA_3 10^{-5} M + cyclic AMP 10^{-5} M, or cyclic AMP 10^{-5} M + theophylline 10^{-5} M. However, slight additive effect was observed at suboptimal concentrations of GA_3 10^{-8} M + cyclic AMP 10^{-6} M, or cyclic AMP 10^{-6} M + theophylline 10^{-5} M. Application of theophylline alone showed nearly 2-fold stimulation of RNAase activity (Table I). These results corroborate the view that GA_3 and cyclic AMP stimulate RNAase activity by acting at a common site. The stimulation of RNAase activity elicited by GA_3 and cyclic AMP was extremely sensitive to the action of Act D and cycloheximide. Both antibiotics inhibited the enhanced RNAase activity to the extent of about 80% (Table III). Thus, RNA and protein synthesis seem necessary for the GA_3 and cyclic AMP promoted activity of RNAase. This was further supported by the fact that both GA_3 and cyclic AMP enhanced the incorporation of ^{3}H-uracil and ^{3}H-leucine into RNA and protein fractions respectively, which was strongly inhibited by Act D and CHI (Tables IV and V). Abscisic acid also suppressed the enhanced RNAase activity evoked by GA_3 and cyclic nucleotide (Table III).

Table IV. Effect of GA_3 and cyclic AMP on ^{3}H-uracil incorporation into RNA fraction

Additions	^{3}H-uracil incorporation	
	cpm/mg protein	Control (%)
Control	1057	100
Act D 50 µg/ml	251	24
GA_3 10^{-5} M	1867	177
GA_3 10^{-5} M + Act D 50 µg/ml	400	38
cAMP 10^{-5} M	1530	145
cAMP 10^{-5} M + Act D 50 µg/ml	350	34

The seeds were germinated for 96 h at 35 ± 2 °C. ^{3}H-Uracil (1 µCi/ml, Spec. act. 6100 mCi/mmole) was added after 48 h germination of seeds. GA_3, cAMP and Act D were added at the beginning of germination.

Table V. Effect of GA_3 and cyclic AMP on ^{3}H-leucine incorporation into protein fraction

Additions	^{3}H-L-leucine incorporation	
	cpm/mg protein	Control (%)
Control	1628	100
CHI 10 µg/ml	265	16
GA_3 10^{-5} M	2667	164
GA_3 10^{-5} M + CHI 10 µg/ml	338	21
cAMP 10^{-5} M	2583	159
cAMP 10^{-5} M + CHI 10 µg/ml	355	22

The seeds were germinated for 96 h at 35 ± 2 °C. ^{3}H-L-leucine (2 µCi/ml Spec. act. 7600 mCi/mmole) was added after 48 h germination of seeds. GA_3, cAMP and cycloheximide (CHI) were added at the beginning of germination.

Fractionation of crude extracts prepared from 96 h old seedlings revealed six isoenzyme bands (R_1–R_6) on acrylamide gels. The stimulatory effect of GA_3 and cyclic AMP on enzyme activity was accompanied by the quantitative and qualitative changes in the isoenzyme pattern of RNAase. Several RNAase isoenzyme bands eluted from the acrylamide gels, revealed enhanced activity in GA_3- and cAMP-treated seedlings (unpublished results). Out of 6 isoenzymes observed in controls, there was a distinct augmentation in the intensity of 4 isoenzyme bands (R_2, R_3, R_5, R_6) by the application of GA_3 and cyclic AMP. In addition, the hormone (GA_3 10^{-5} M) treatment resulted in the appearance of 5 new minor bands (R_A, R_B, R_C, R_D, R_E). The action of GA_3 was mimicked by cyclic AMP (10^{-5} M), since it also caused the appearance of 4 identical minor bands in addition to the augmentation of the pre-existing activity bands (R_A, R_B, R_C, R_E) (Figure 2). These studies suggested a similar response of RNAase isoenzymes to GA_3 and cyclic AMP. Addition of Act D along with GA_3 or cyclic AMP completely abolished the appearance of all the newly formed minor activity bands. Thus, only 2 RNAase isoenzymes (R_1, R_3) were observed when Act D was added to GA_3- and cyclic AMP-treated seedlings. Cycloheximide caused an inhibitory response similar to that observed with Act D. Addition of ABA to seedlings raised on GA_3 or cyclic AMP medium abolished the appearance of all the newly formed isoenzymes and also the 3 pre-existing activity bands (R_2, R_4, R_5). The action of ABA resembled the inhibitory response of Act D and CHI, except that the R_6 band persisted in addition to R_1 and R_3 (Figure 2). The modulation of RNAase isoenzymes by GA_3 and cyclic AMP in germinating cowpea seeds could be at the transcriptional and translational levels. However, the possibility of activation, inhibition or aggregation cannot be ruled out unequivocally unless the de novo synthesis of RNAase is established in this system.

Zeitgeber Induced Modulation of Activity Patterns in Nocturnal Mammals (Chiroptera)[1]

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Summary. Changing the L:D intensity ratio of a synchronizing light-dark regime leads to characteristic modulations of the activity pattern of 5 Chiroptera species. These modulations fit the predictions of WEVER's oscillator model.

WEVER's oscillator model[2–5] makes the most explicit predictions about the characteristics of circadian systems under different Zeitgeber conditions, of all hitherto developed models of biological 24-hour-periodicity (for review see[6]). According to one of these predictions, increasing L:D intensity ratio and decreasing mean intensity of illumination of a synchronizing light-dark-

[1] Supported by a grant from the Deutsche Forschungsgemeinschaft (No. Er 59, 1–4).

[2] R. WEVER, Kybernetik *1*, 139 (1962).

[3] R. WEVER, Kybernetik *1*, 213 (1963).

[4] R. WEVER, Kybernetik *2*, 127 (1964).

[5] R. WEVER, *Circadian Clocks* (Ed. J. ASCHOFF; North Holland Publ. Company, Amsterdam 1965).

[6] A. JOHNSSON and M. G. KARLSSON, J. theor. Biol. *36*, 153 (1972).

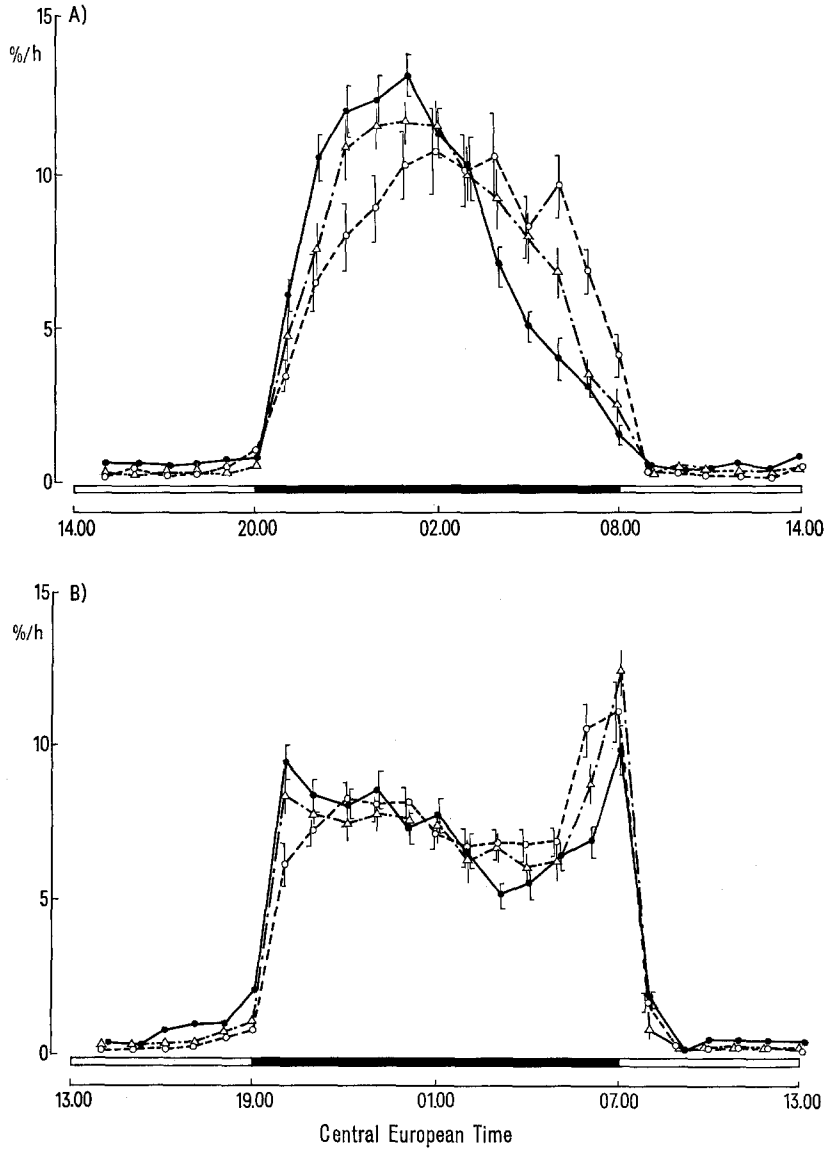

Fig. 1. The effect of L:D-intensity-ratio on the activity pattern of bats. Mean values and standard errors as computed over all $n \times n'$. A) *Phyllostomus discolor*. +- - -+, L:D = 2.5×10^{1}:10^{-2} lx; △- · -△, L:D = 2.5×10^{1}:10^{-4} lx; ●—●, L:D = 2.5×10^{1}:10^{-6} lx). B) *Molossus molossus*. +- - -+, L:D = 5×10^{1}:4×10^{-2} lx; △- · -△, L:D = 5×10^{1}:4×10^{-4} lx; ●—●, L:D = 5×10^{1}:4×10^{-6} lx.

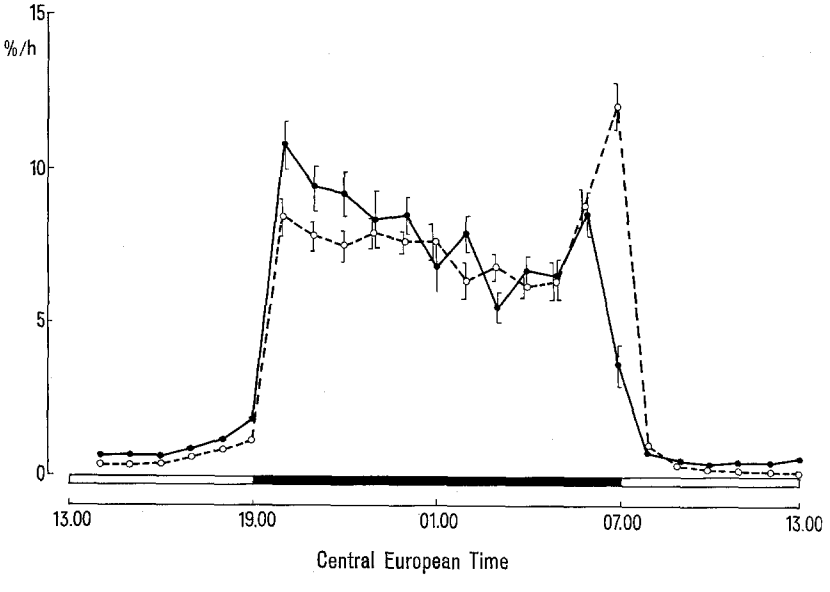

Fig. 2. Modulation of the activity pattern of *Molossus molossus* by reduced illumination intensity during the light-phase of a light-darkness-regime 12:12 h. +- - -+, L:D = 5×10^{1}:4×10^{-4} lx; ●—●, L:D = 3×10^{-2}: 4×10^{-4} lx.

regime should induce a modulation of the activity pattern (shape of oscillation) by advancing the maximum and giving rise to a steeper increase of activity. Thus, bimodal activity patterns of the alternans-type[7] may finally change into the bigeminus-type. Since this model deduction has scarcely been tested on an experimental basis, we carried out experiments with 5 species of bats.

Species used for activity measurements under different light-darkness conditions were *Myotis myotis* ($n = 9$; duration of each registration period $n' = 10$ days), *Rousettus aegyptiacus* ($n = 5$; $n' = 20$), *Phyllostomus discolor* ($n = 4$; $n' = 10$), *Molossus ater* and *Molossus molossus* ($n = 3$; $n' = 12$–17 days). In order to determine the gross locomotory activity of the bats, we used an electroacustic registration equipment according to BAY[8], climbing bars mounted upon micro-switches for *Rousettus* respectively, and an ELMEG multi-channel digital printer. Every hour compiled activity data were calculated as percentage of the respective total amount of activity per day. Thus, it was possible to determine comparable general activity patterns according to different strengths of Zeitgeber.

All 5 Chiroptera-species tested show modulations of the activity pattern caused by different Zeitgeber conditions, as predicted by WEVER[4,5]. The activity pattern of *Phyllostomus discolor* (Figure 1), recorded at 3 different Zeitgeber conditions, serves as an example showing the deformation of an unimodal pattern. The activity pattern of this bat is nearly symmetric when there is only a little difference between L (25 lx) and D (10^{-2} lx). With increasing strength of Zeitgeber, the maximum is evidently advanced, combined with a steeper increase of activity at the beginning of the dark period. The unimodal activity pattern of the African fruit bat, *Rousettus aegyptiacus*, also yields similar tendencies of pattern deformation.

The bimodal activity patterns of the neotropical *Molossus* species are affected by the increase of range of L:D oscillation, so that there is a gradual decrease of the second maximum, which appears just before the end of the dark period, and an increasing accentuation of the first maximum. This is illustrated for *M. molossus* in Figure 1, B. Whereas there is no complete change from alternans type to bigeminus type in the activity pattern of this species, *Molossus ater* clearly changes its activity pattern to bigeminus type. *Myotis myotis* advances the second maximum of its bigeminus pattern, appearing in the second third of the dark period with increasing L:D-intensity ratio. The main maximum, appearing just after beginning of the dark period, reaches the highest level at the greatest Zeitgeber strength.

There are also changes of the activity pattern if the average intensity of illumination of the LD 12:12 is reduced by decreased brightness during the L-phase (Figure 2). *M. molossus* shows an activity pattern of alternans type in LD 51: 4×10^{-4} lx, and an activity pattern of bigeminus type in LD $3 \times 10^{-2} : 4 \times 10^{-4}$ lx.

These results, though in remarkable agreement with the very explicit deductions of WEVER's oscillator model, cannot be taken as general proof for this model. Competing models[6], however, have to be tested whether they are able to predict similar Zeitgeber-induced modulations of activity patterns. The results indicate in addition that 'the activity pattern' of a species does not exist. There are only patterns of species and individuals under certain conditions[9,10]. In conclusion it is essential to have the same methods of registration and comparable Zeitgeber conditions in order to compare different activity patterns.

[7] J. ASCHOFF, Naturwissenschaften *44*, 361 (1957).
[8] F. A. BAY, unpubl. Diss., Tübingen 1975.
[9] J. ASCHOFF and K. HONMA, Z. vergl. Physiol. *42*, 383 (1959).
[10] H. G. ERKERT, Oecologia *14*, 269 (1974).

Isoenzyme Patterns in Androgenic, Haploid *Datura meteloides* (Solanaceae)

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Summary. Leaf tissue isoenzyme patterns of androgenically derived, haploid plants of *Datura meteloides* were compared with patterns from diploid parental plants. No isoenzyme patterns unique to the haploid plants were detected among 5 enzymes studied.

Haploid angiosperm plants have been observed to occur sporadically through accident or experimental design[1]; but only recently has tissue culturing of androgenic plants provided a consistent source of experimental, haploid angiosperm material[2,3]. The potential utility of this technique for isolating auxotrophic angiosperm mutants, generating uniformly homozygous breeding stocks and identifying useful somatic genetic markers was quickly appreciated. Some of these prospects have already been realized; e.g., regeneration of diploid tobacco from colchicine treated, androgenically derived cells[4] and the identification of malate dehydrogenase as a useful marker in somatic cell genetics[5]. The present report is a genetic comparison of androgenically derived haploid plants with their diploid parental stocks utilizing selected isoenzymes as genetic markers.

Anthers from plants of *Datura meteloides* A. DC. occurring naturally at the Rancho Santa Ana Botanic Garden, Claremont, CA, were aseptically cultured using reported proceedures[6,7]. The ploidy level of putative haploids was established by chromosome counts of root tip cells (with the result that $2n = 12$) and by measuring stomate dimensions on haploid and parental plants, a parameter known to be sensitive to ploidy level[8]. The stomate dimension ratio of diploid to putative haploid plants was 1.4. Samples for electrophoresis were prepared by grinding fresh leaf material in distilled water, clearing by centrifugation ($5000 \times g$) and electrophoresing the opal-

[1] G. KIMBER and R. RILEY, Bot. Rev. *29*, 480 (1963).
[2] J. P. NITSCH and C. NITSCH, Science *163*, 85 (1969).
[3] S. NARAYANSWAMY and L. P. CHANDY, Ann. Bot., Lond *35*, 525 (1971).
[4] L. G. BURK, G. R. GWYNN and J. F. CHAPLIN, J. Heredity *63*, 355 (1972).
[5] P. S. GANAPATHY and J. G. SCANDALIOS, J. Heredity *64*, 186 (1973).
[6] J. P. NITSCH, Phytomorphology *11*, 2 (1961).
[7] K. C. ENGRILD, I. LINDE-LAURSEN and A. LUNDQVIST, Hereditas *72*, 331 (1972).
[8] A. F. BLAKESLEE, Am. Nat. *75*, 117 (1941).