Differences in the mycelial growth rhythms in a population of *Sclerotinia fructigena* (Pers.) Schröter

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Summary. Of 196 strains of *Sclerotinia fructigena* 181 grew rhythmically in light-dark cycles, while 30 isolates also exhibited rhythms under constant conditions, which indicates an endogenous periodicity. Among the latter 4 strains were found with well-expressed circadian rhythms. The results are thought to be compatible with an individual genetic creation of rhythms in this population.

Rhythmic growth and fructification are often found in fungal cultures and were recognized as early as 1898 in laboratory mycology[1]. In the following years a variety of forms were observed and studied. The investigations about this growth type mostly dealt with physiology, with the main emphasis on spontaneously oscillating mutants, the 'clock' mutants[2,3].

Not very much, however, is known about this type of growth and reproduction in nature, where it is represented by obvious structures like the 'ring rot' of various fruits, the diurnal rings of the moulds or the 'fairy rings' of the Agaricales. Some studies have dealt with these fairy rings, which are regarded as a form of periodic fruiting[3–5].

To obtain information about the distribution of growth rhythms in natural populations, a fungus was searched for which was known to show rhythmic activity and which was easy to isolate and to handle in order to allow examination of a sufficient number of samples. *Sclerotinia fructigena*, the cause of the ring rot (or brown rot) of fruit proved suitable in these aspects. Its imperfect state forms sporodochia on infected fruits. These sporodochia are combined to form rings which are formed in light-dark-cycles and which are regarded as being dependent on a diurnal (light-dark-induced) mycelial growth rhythm as was found in cultures of this fungus by Hall[6]. The investigations of Molz[7] and Hall[6] on the growth of this fungus provided a basis for these experiments.

Materials and methods. Isolates of *Sclerotinia fructigena* (Pers.) Schröter were obtained from apples, pears and prunes grown in private orchards in Berlin (West). Isolation was carried out by transferring conidia from sporodochia to artificial media in Petri dishes. No attempt was made to obtain genetically homogenous material. The fungus was grown on the following medium: 50 ml apple juice, 20 g malt extract, 20 g agar, 950 ml destilled water, or on a growth and experimental medium with 20 g malt extract and 20 g agar in 1 l tap water.

The cultures were grown in light-dark-cycles of 10 h light and 14 h dark (LD), in continuous dark (DD) or in continuous light (LL), at 27 °C. As defined by Bünning[8] light-dark induced rhythms are called diurnal; those expressed in DD are called endogenous; while circadian rhythms are endogenous, show a period of about 24 h and are temperature-compensated.

Growth was measured every 3 days during the period of continuous growth of the colony. The linear growth rate was calculated by regression analysis. The period τ of the growth rhythm was calculated as the quotient of mean band size and linear growth rate:

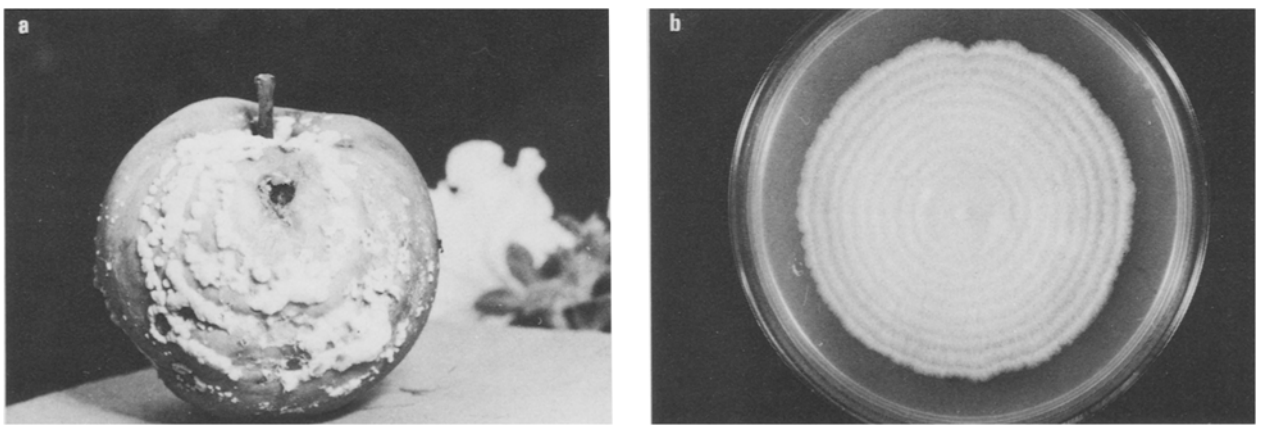
$$\tau = \frac{\text{mean size of the rings (mm)}}{\text{linear growth rate (mm} \cdot \text{day}^{-1})}$$

Results. The figure gives the typical rings of the sporodochia on an infected fruit (a) and a culture of the mycelium showing the corresponding mycelial growth rhythms in LD (b). These cultures had a mean rate of colony growth of 5.39 ± 0.08 mm·day^{-1} at 27 °C (mean of all values obtained). The colonies grown in DD were not measured in order not to interrupt growth conditions. When related to the reduced colony size, the linear growth rate in DD is about 80% of the LD-values. Similar values were obtained by Willetts[9]. Thus, all the colonies showed sufficient growth in culture to allow testing for their rhythms.

Figure b shows a culture with growth rhythms expressed in concentric mycelial bands. These concentric rings or bands are due to the diurnal, i.e. light-dark-induced development of aerial mycelia in the cultures. As in other fungi and in

Table 1. Expression of rhythms by the isolated strains of *Sclerotinia fructigena* in LD and DD light regimes at constant temperature (27 °C)

Light regimes	No. of isolates growing rhythmically	No. of isolates growing uniformly	Total No. of isolates
LD 10:14	181	15	196
DD	30	166	



a Apple infected with the brown rot fungus *Sclerotinia fructigena* exhibiting typical diurnal rings of sporodochia. *b* Petri dish culture of *Sclerotinia fructigena* grown in light-dark-regimes (10:14 h) and exhibiting typical growth bands.

Table 2. The endogenous periods of the four strains exhibiting a clearly evaluable circadian rhythm. Growth on malt-extract medium in permanent dark (DD) at the given temperatures

Isolate No.	Endogenous period at °C (h)			Total mean value
	22 °C	27 °C	32 °C	for each strain
24	22.55 ± 0.76	21.68 ± 0.84	24.72 ± 1.10	22.40 ± 0.56
26	22.23 ± 0.60	22.87 ± 1.13	24.39 ± 0.35	23.06 ± 0.50
79	24.48 ± 1.56	24.51 ± 0.67	23.52 ± 0.70	24.13 ± 0.51
88	21.96 ± 0.84	22.60 ± 0.41	22.08 ± 1.55	22.29 ± 0.48
Total mean values for each temperature	22.80 ± 0.52	22.84 ± 0.41	23.49 ± 0.52	

Means of 5 replicas ± SD.

the typical clock mutants[10,11] bands or zonations are formed by an alternation of thin and densely branched mycelia. The majority of the isolates (181 out of 196, see table 1) exhibited this growth type in LD. The bands were synchronized to the LD-cycles as was found previously by Molz[7]; the rhythm, however, did not start before 3–4 days after inoculation[9]. The morphogenesis of the mycelial growth bands was found to be similar to that described by Kubicek and Lysek[12].

More interesting were those strains which also grew rhythmically in DD at a constant temperature. As is seen in table 1, these formed only a minor part of the isolates. In addition, some of them had unclear bands, which prevented exhaustive testing. Four of the remaining strains which formed clear bands with periods near to 24 h were subjected to temperature tests. The result is seen in table 2. The endogenous period of these 4 strains is obviously temperature-compensated, a crucial condition for circadian rhythms. The temperature coefficient is 1.031 for the period (τ) and +0.97 for the frequency (which gives the time-dependent alteration). Table 2 also shows that the strains exhibited significantly different endogenous periods, which may show that these periods are inherited[8]. In LL the bands were suppressed, which also characterizes circadian rhythms. This gives evidence that in the population of *Sclerotinia fructigena* circadian rhythms occur in a basic form as mycelial growth rhythms.

Discussion. The experiments give evidence that besides the light-dark-induced rhythm, other types of rhythms occur in the analyzed population of *S. fructigena*. These are thought to be caused endogenously, but normally not exhibited because of the dominating light-dark diurnal rhythms under natural conditions. The diurnal rhythm is undoubtedly advantageous for the fungus since the bands formed are capable of producing conidia. The concentration of the conidiophores on the mycelial bands allows the fungus to combine them to sporodochia and to enhance their total number[3]. Under the cover of this exogenous, light-dark-induced rhythm other forms are hidden, but, as the experiments show, nevertheless exist. They are obviously caused by spontaneous mutations. The occurrence of spontaneously caused clock-mutations has been shown by Lysek, Hohmeyer and Veltkamp (in preparation) in *Podospora anserina* and they are to be expected in natural populations. Since they are neither advantageous nor disadvantageous, they are not eliminated by selection and thus maintained in the population. Although these results with *S. fructigena* might represent conditions in other fungal populations, different types of rhythms might be found with other organisms. Fungi are especially well suited for these investigations, since many of them do not show distinct rhythms, especially no circadian ones. In other eucaryotic organisms with well-developed circadian rhythms, however, other forms of rhythms might be disadvantageous and therefore quickly eliminated.

On the other hand, the results obtained indicate that mutations affecting rhythmic behavior also occur in nature and, as pointed out by Sweeney[13], might provide the organism with an essential plasticity or adaptability in its rhythmic behavior.

To obtain a broader basis for the discussion of these problems, further investigations are necessary.

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High resolution of heterochromatin of *Drosophila melanogaster* by distamycin A

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Summary. A DNA-binding AT-specific antibiotic, distamycin A, was used as inhibitor of the condensation process of the heterochromatic regions in *Drosophila melanogaster* embryonic cells. By this treatment the structural organization of heterochromatin at interphase is preserved until metaphase. The different patterns observed are interpreted as chronological steps in the condensation process.

Heterochromatin of *Drosophila melanogaster* is clearly defined genetically, cytologically and at a molecular level: satellite DNA sequences located in this chromosome portion have revealed a high repetitivity of AT base pairs (BP)[2]. Since the most evident property of heterochromatin is that of remaining in a deeply-staining condensed state