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

during the entire cell cycle, a modification of this structural trait may throw light on unknown characteristics of this part of the chromosome. Recently DNA ligands, distamycin A[3], Hoechst 33258[4], and DAPI[5] have been discovered which bind specifically to AT-rich double-stranded DNA without intercalation, therefore inhibiting the spiralization of the heterochromatic regions. In order to analyze the condensation of the heterochromatin in *Drosophila melanogaster*, distamycin A was added to cultures of embryonic cells. By this treatment the structural organization of heterochromatin at interphase is preserved until metaphase and the chronological steps of the condensation process can be examined.

Material and methods. *Drosophila melanogaster* embryonic cells were obtained from eggs laid by females of the Varese wild strain over a period of 12 h. Cells, isolated by homogenization from eggs dechorionated in 3% NaClO, were cultured at 25 °C for 5 h in D 225 medium of Echalier and Ohanessian[6], supplemented with 18% fetal bovine serum and with distamycin A hydrochloride (Boehringer, Mannheim) to a final concentration of 100 µg/ml. 1 h before fixing, colchicine (5 µg/ml) was added. Preparations were obtained by a standard air-drying technique. Slides were stained with Giemsa (4% Merck Giemsa in M/100 Sorensen's buffer, pH 7) for 45 min, rinsed in deionized water and mounted in euparal. For fluorescence analysis a few slides were treated with a 0.5% solution of quinacrine dihydrochloride and observed at a Zeiss photomicroscope equipped with a HBO 200 mercury vapour lamp, a 1.5 mm BG 12 exciter filter and a 500 nm barrier filter.

Results. The effect of distamycin A treatment (100 µg/ml) for 5 h on embryonic cells of *Drosophila melanogaster* is reported in the Table. Metaphases displaying a normal compact condensation and a standard fluorescence (Q-banding) pattern at the level of the heterochromatin were present (fig. 1, a). However, different degrees of undercondensation of the quinacrine-bright heterochromatin were observed in a considerable proportion of metaphases. Affected metaphases were classified as follows:

1. Metaphases displaying an elongation of the heterochromatin, essentially in the Y and in the 4th pair along the whole length, and in the centromeric regions of the 3rd pair, the 2nd pair being unaffected (fig. 1, b–e). This pattern of undercondensation confirms the overlapping between distamycin-sensitive regions and chromosome sections cytologically and genetically defined as heterochromatic, if

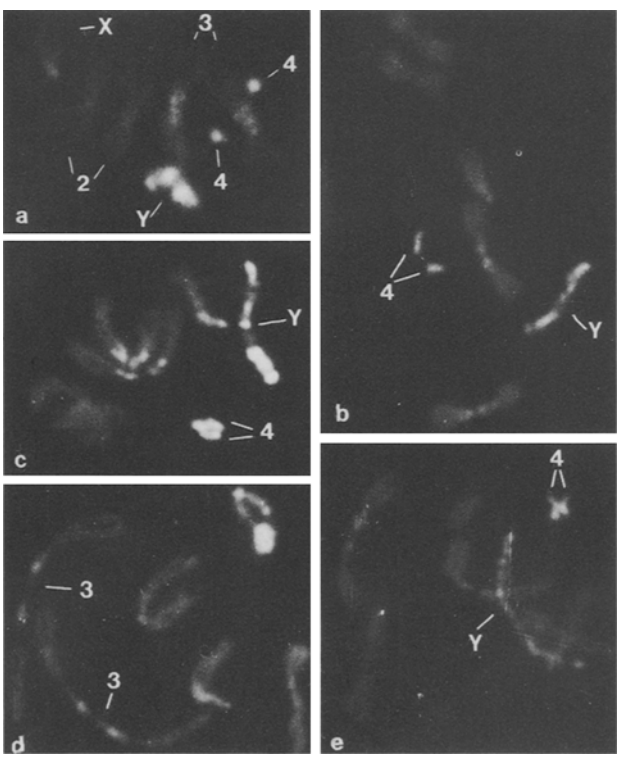

Figure 1. Metaphases of *Drosophila melanogaster* embryonic cells treated with distamycin A (100 µg/ml for 5 h) and stained with quinacrine. *a* Male metaphase showing normal fluorescence pattern: bright fluorescence in the Y, in 4th pair, in the centromeric regions of 3rd pair and weak fluorescence in proximal region of the X. *b–e* Metaphases showing elongation of heterochromatin. Y chromosome exhibits different degrees of decondensation, intermediate (b), high (c) and drastic (e). Centric heterochromatin of 3rd chromosomes is drastically decondensed in (d). 4th chromosomes are decondensed along their whole length (b, c, e). 2nd pair is unaffected in all metaphases. ×2400.

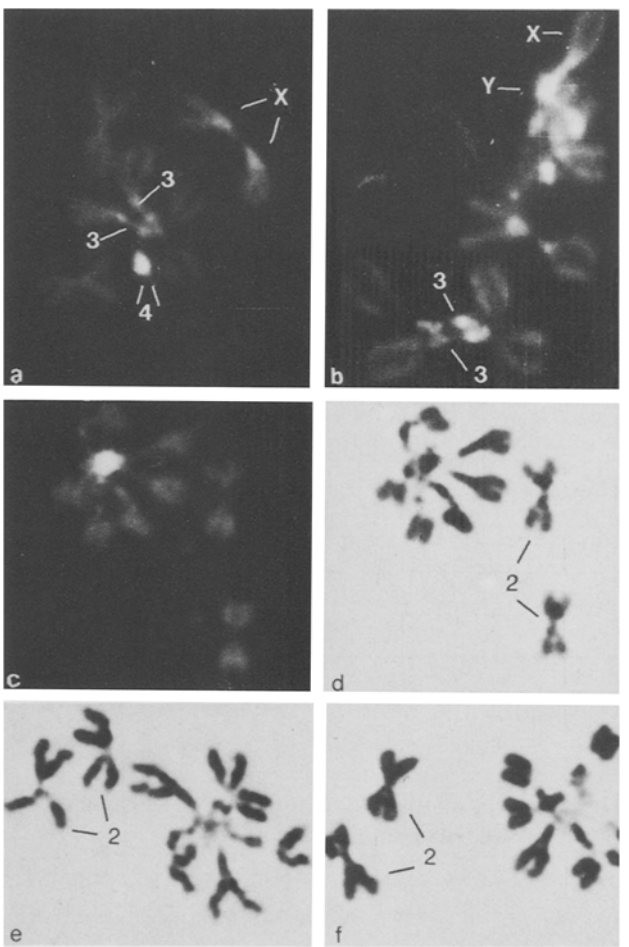

Figure 2. Metaphases of *Drosophila melanogaster* embryonic cells treated with distamycin A and stained with quinacrine. *a, b* Metaphases showing homologous pairing between undercondensed heterochromatin (a). A thin filament of decondensed heterochromatin connects X chromosomes. Pairing between centric regions of 3rd chromosomes and coalescence of 4th pair in 1 fluorescent mass. Pairing between centric regions of 3rd chromosomes and fusion between heterochromatin of the X and whole Y completely decondensed (b). *c–f* Ectopic pairing between all undercondensed heterochromatic regions. *c, d* Same metaphase stained with quinacrine (c) and Giemsa (d). Coalescence of heterochromatin (identified by bright fluorescence) into a 'chromocenter-like' structure connecting normally condensed euchromatic arms. 2nd pair, unaffected by distamycin A, is not included in this structure. ×2400.

they are quinacrine-bright and AT-rich. The validity of this assumption is confirmed by the fact that centromeric heterochromatin of the 2nd pair (not quinacrine-bright) is insensitive to the effect of distamycin A. The heterochromatin of each chromosome in *Drosophila melanogaster* has a unique quantitative and qualitative identity; by in situ hybridization[7], the absence from the centromeric heterochromatin of the 2nd pair of the 1.672 g/cm^3 DNA satellite (exclusively composed of AT BP), and the presence of the 1.705 g/cm^3 and of the 1.686 g/cm^3 DNA satellites (both characterized by the presence of GC BP as well), were demonstrated. Distamycin A is clearly inactive where GC BP are predominant or strategically placed.

2. Metaphases revealing a pairing between homologous undercondensed heterochromatic sections (fig. 2, a and b), which tend to stay in close proximity even at this stage of mitosis, when the somatic pairing, characteristic of *Drosophila* chromosomes, has usually disappeared. A fusion or coalescence of heterochromatin of homologous chromosomes (particularly sex chromosomes) may be evident.

3. Metaphases with close association of undercondensed heterochromatin (fig. 2, c–f). 'Chromocenter-like' structures connecting normally condensed euchromatic arms are evident, the unaffected 2nd pair being a marker of the metaphase stage. We designate this unspecific association between heterochromatic regions as 'ectopic pairing'[8]. By means of distamycin A, which prevents the normal condensation, the physical contact between the associated heterochromatin, typical of interphase and presumably due to similarity in base sequences[9] is preserved until the metaphase.

Discussion. On the basis of the frequencies of the different patterns observed, which are an expression of the progressive degree of spiralization, a sequence in the condensation of the heterochromatin may be suggested. Ectopic pairing, homologous pairing and elongation could reflect chronological steps in the continuous spiralization process. In fact heterochromatin in the interphase may be closely and unspecifically associated; as the condensation proceeds, the pairing becomes less tight and restricted to homologous regions, gradually disappearing at the start of mitosis.

These data may be compared with similar results obtained after treatment with Hoechst 33258 of *Drosophila melanogaster* gangliar cells[10] and of *Drosophila nasuta* embryonic and gangliar cells[11]. Different degrees of decondensation have been reported for the different chromosomes of *Drosophila melanogaster*[10] (the 2nd pair being unaffected also in this case), but the sequence of the condensation process has not been indicated. In *Drosophila nasuta*[11] a difference has been reported between the 2 types of cells considered, as regards response to the agent, since the embryonic cells seem to be more sensitive. Thus they offer a better potential for analysis; however, this is limited because there is less genetical knowledge of this species.

In conclusion, the high degree of resolution in the present analysis of the condensation process may be attributed to the decondensing agent (distamycin A) and to the cell system (embryonic cells) used.

Effect of distamycin A (100 µg/ml) treatment on heterochromatin of embryonic cells of *Drosophila melanogaster*

Hours of treatment	No. of metaphases analyzed	Normal	Affected Elongation	Homologous pairing	Ectopic pairing
5	937	524 (55.92%)	277 (29.56%)	75 (8.00%)	61 (6.51%)

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Development of adenocarcinomas after transplantation of rat glandular stomachs treated in vitro with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)[1]

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Summary. Glandular stomachs of fetal and newborn Wistar rats were transplanted s.c. after treatment in vitro with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) at concentrations of 10, 5 and 1 µg/ml for 2 h. Eleven adenocarcinomas developed from 118 MNNG-treated transplants, whereas no adenocarcinomas developed from 28 untreated transplants. The incidence of adenocarcinomas in fetal glandular stomach (9/46) was significantly different ($p < 0.01$) from that in glandular stomach of newborn rats (2/67). Various types of mesenchymal tumors also developed from untreated (9/28) and MNNG-treated (20/118) transplants.

Although MNNG is a potent carcinogen and is known to induce gastric carcinomas in rats[1], there have been few studies in vitro on its carcinogenetic effect on the glandular stomach epithelium[3,4], probably owing to the difficulty of culturing epithelial cells of the glandular stomach[5,6]. We have been studying organ cultures of newborn rat glandular stomach[7]. Using the organ culture technique in combination with the transplantation method, we succeeded in transforming glandular stomach epithelium after treatment with MNNG in vitro.

Materials and methods. The glandular stomachs of fetuses (16–20 days) and newborn rats (within 72 h after birth) of