question until such comparisons can be made on a larger number of different plant growth and behavioral phenomena.

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Neurospora Circadian Rhythms in Space: A Reexamination of the Endogenous-Exogenous Question

Abstract. *To test the functioning of circadian rhythms removed from periodicities of the earth's 24-hour rotation, the conidiation rhythm of the fungus* Neurospora crassa *was monitored in constant darkness during spaceflight. The free-running period of the rhythm was the same in space as on the earth, but there was a marked reduction in the clarity of the rhythm, and apparent arrhythmicity in some tubes. At the current stage of analysis of our results there is insufficient evidence to determine whether the effect seen in space was related to removal from 24-hour periodicities and whether the circadian timekeeping mechanism, or merely its expression, was affected.*

Daily rhythmic patterns of plants and animals have been recognized from earliest times. In 1729 DeMairan (*1*) published the first demonstration that the 24-hour light-dark cycle was not essential to the leaf movement rhythm of a plant (probably *Mimosa pudica*). Since that time, the question of how circadian rhythms can persist in the absence of obvious environmental time cues has continued to interest biologists. Before the end of the last century two schools of thought had developed: investigators such as Darwin (*2*) proposed that daily rhythms were inherent, while others such as Pfeffer (*3*) felt that these rhythms were responses to subtle daily changes in the environment. The middle of this century saw a flowering of interest in biological rhythm research and since that time the major features of circadian clocks have been well characterized (*4*).

Most investigators studying circadian rhythms feel that the preponderance of evidence indicates that daily timekeeping results from cellular processes (biological clocks) which use environmental time cues (especially light and temperature) for entrainment to the 24-hour day. The non–24-hour period of these rhythms, as expressed in the absence of light and temperature cycles, does not match any known geophysical periodicity. This circadian period has been demonstrated to be genetically determined (*5*), and cellular timing can be modified by drugs (*6*). Further, there is no persuasive evidence that eliminating geophysical time cues affects circadian rhythms. For example, Hamner *et al.* (*7*) showed that circadian rhythms of hamsters (*Mesocricetus auratus*, fruit flies (*Drosophila*), bean plants (*Phaseolus vulgaris*), and fungi (*Neurospora crassa*) continued even when the organisms were placed at the South Pole on a table with a daily rotation in the direction opposite to that of the earth's spin.

Another opinion was championed by Brown (*8*), who argued that none of the experiments which showed the endogenous nature of circadian rhythms was conclusive. Brown proposed that because of the earth's daily rotation on its axis, there were subtle 24-hour fluctuations in many geophysical parameters which experimenters could not or did not eliminate, and these provided temporal information. Through a process he called autophasing, organisms could utilize these 24-hour signals to time rhythms with non–24-hour periods.

Since on the earth's surface there is no way of eliminating all potential 24-hour periodicities, we designed an experiment which was flown on the Spacelab 1 flight. Because the spacecraft orbited the earth every 90 minutes and was staffed continuously in shifts around the clock, 24-hour time cues were severely attenuated, if not eliminated. We measured the conidiation rhythm of the band strain of the common fungus *Neurospora crassa* (*9*) in constant darkness. The genetics and biochemistry (*10*) of the *Neurospora* circadian system are well characterized and the simplicity of conducting experiments with *Neurospora* obviated many potential problems of research in space. We report here the preliminary results of our experiment.

To monitor rhythmicity, we used race tubes containing a Vogel's salts (*11*) and acetate medium (*12*). Cultures grown on the earth in constant bright light display no rhythmicity. However, if the cultures are transferred from constant light to constant darkness, a distinct rhythmic pattern is evident, as can be seen in Fig. 1. The white patches in each tube indicate times at which conidiation (vegetative spore formation) occur. The interval between patches of conidiation is the circadian period, and for the band strain this period is 21.5 to 22.0 hours. Although the growth rate of *Neurospora* increases as the ambient temperature is raised, several authors have shown that the free-running period is relatively unaltered by temperature (*13*).

For the spaceflight, cultures were grown in race tubes in bright light at 26°C for 2 days and then transferred to constant darkness on the day before the launch. Twenty-four tubes were placed in a foam package which also contained a high-energy radiation dosimeter and a solid-state ambient temperature recorder. About 12 hours before launch the package was transported from our laboratory at the Kennedy Space Center to the space shuttle, where it was stowed in a mid-deck locker. On the seventh day of the flight, the package was removed from the locker and each tube examined in fluorescent light (*14*). It took less than 30 minutes to mark the growth fronts on the tubes, and then the package was restowed in the locker in constant dark-

ness for 86 more hours. Within 2 hours after the shuttle landed at Edwards Air Force Base, the package was removed and the race tubes photographed (Fig. 2).

The pattern of the spaceflight cultures shown in Fig. 2 was visibly different from that of cultures grown on the earth in three respects: (i) there was a much greater variation in growth rates among the tubes, (ii) there was an increase in variance of the circadian period, and (iii) the clarity of banding, which probably reflects the amplitude of the circadian rhythm, was considerably reduced. In fact, by the seventh day of spaceflight, rhythmicity had apparently damped out in about one-fourth of the cultures. However, after the cultures were marked by the payload specialist on day 7 and returned to constant darkness for 86 hours, robust rhythmicity was evident in all of the tubes. Unfortunately, there were fairly large changes (21.5° to 26.9°C) in the package temperature during the spaceflight. Because of this nonuniform temperature, we calculated the free-running period by including the effect of ambient temperature on growth rate. The average free-running period for those cycles which could be measured for the first 167 hours in space was 22.8 ± 0.3 (mean ± standard error) hours and during the last 86 hours the period was 22.7 ± 0.9 hours.

The clear rhythmicity seen in all cultures after the tubes were marked proves that the conidiation rhythm can persist in space. Although the free-running periods were not significantly different from those measured on the ground (*15*), there was a striking loss of rhythm amplitude in all of the tubes during the first 7 days in space. There are several possible explanations for these results.

First, it could be that the clock mechanism functions normally in space, but the expression of the rhythm is obscured by microgravity. For example, gas movement in microgravity is reduced because of the absence of convection, and this could result in the local buildup of CO_2 levels. It has been shown that the conidiation rhythm is sensitive to CO_2 (*16*). The resumption of rhythmicity after the tubes were marked could have been due to gas mixing resulting from movement of the tubes.

Our results do not exclude the possibility that the timekeeping mechanism itself is affected by spaceflight. The exogenous clock hypothesis predicts that the absence of daily geophysical time cues would alter circadian clock function

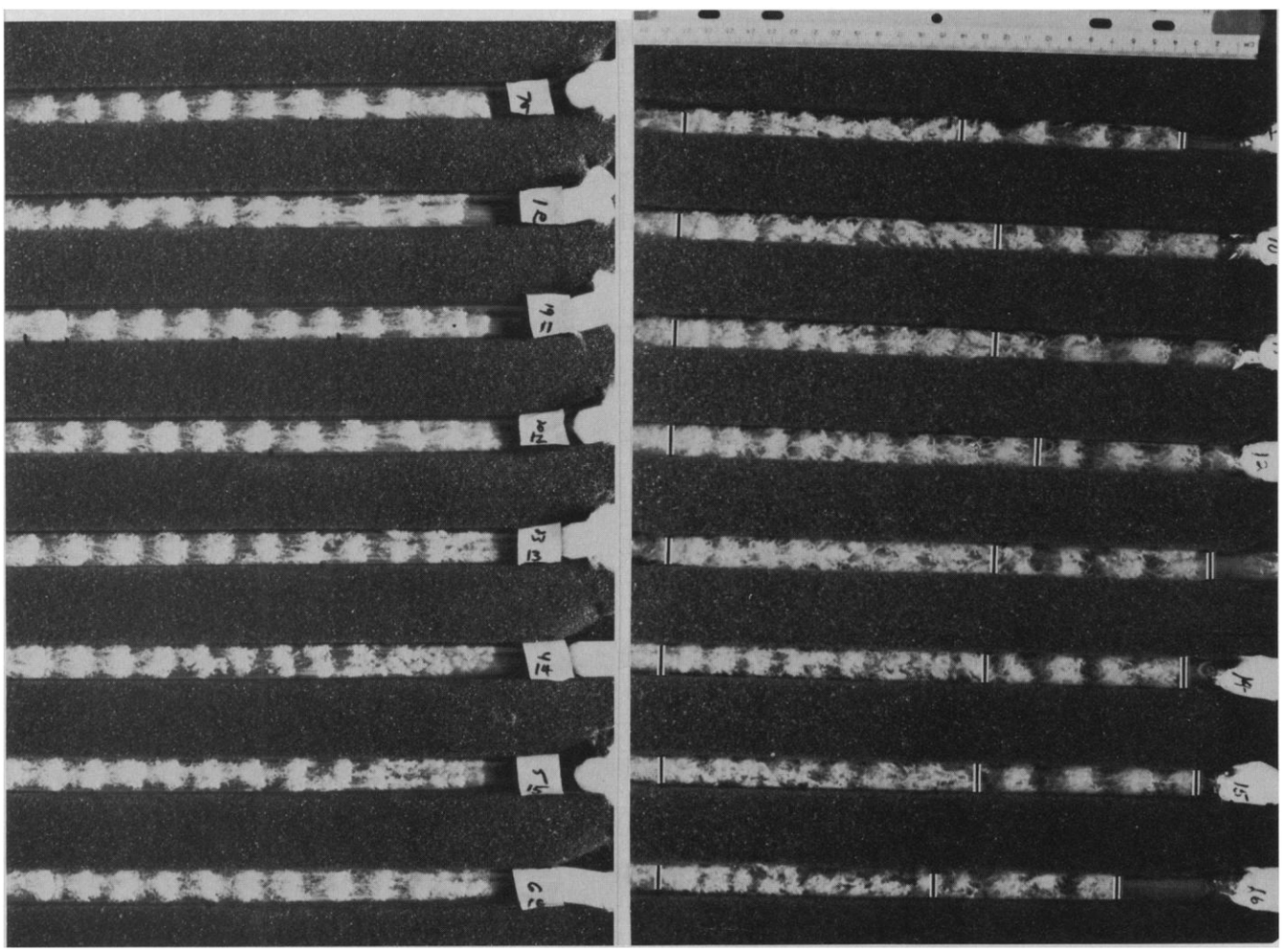

Fig. 1 (left). Circadian rhythm of conidiation of the band strain of *Neurospora crassa* monitored on the earth. Each tube was inoculated on the left end and the *Neurospora* grow toward the opposite end. The tubes were maintained for 2 days in constant light at 27°C and then transferred to constant darkness for 9 days. They were then placed in constant light for an additional day before photography. Ten conidiation bands are present in each tube. Fig. 2 (right). Conidiation pattern of eight of the tubes that were flown aboard the space shuttle. On 27 November 1983, indicated by the black mark on the left end of each tube, the cultures were placed in constant darkness. Launch occurred on 28 November. The tubes were incubated in constant darkness until 5 December, at the time indicated by the second mark on each tube. At this time the package was opened in fluorescent light and the growth fronts of the tubes were marked. After less than 30 minutes the package was returned to constant darkness until landing on 8 December 1983. Two hours after landing the tubes were photographed and marked for the third time.

by a change in either period or amplitude. The conidiation rhythm would then damp out because the clock is not capable of self-sustained oscillations and must have periodic time cues for rhythmicity to continue. The light pulse that the tubes received while they were marked could reinitiate rhythmicity, but if monitored for additional cycles, rhythmicity would again damp out.

There is an alternative to the exogenous clock hypothesis which could also result in a degradation of circadian clock function in space, namely, that gravity influences the cellular timekeeping mechanism. The fact that electric fields have been reputed to affect circadian rhythms (*17*) suggests that there may be some specific spatial organization of circadian clocks. The hypergravity exposure accompanying launch (8 minutes of up to 3 *g*) may have affected clock function. Alternatively, the absence of gravity may have produced some biophysical alteration in a state variable of the fundamental oscillating system. After the tubes were marked, circadian rhythmicity could have resumed if the process of removing and then restowing the package produced sufficient acceleration to initiate circadian oscillations.

Finally, it is possible that the gradual fluctuations in ambient temperature could have affected either cellular timekeeping or its expression, since the mean temperature was lower before the tubes were marked than after. Although we and others (*13*) have observed rhythmicity over the range 21.5° to 26.9°C, weightlessness may result in some unusual temperature sensitivity.

As noted above, the overwhelming weight of previous experimental evidence, as well as the results of this experiment which show that the circadian period of the *Neurospora* conidiation rhythm is normal in space, strongly argue against the exogenous clock hypothesis. However, with the cause of the loss of rhythm amplitude during the first week in space unresolved, some minor role of exogenous factors cannot be entirely excluded.

The study of how circadian clocks function in space is just beginning. This experiment raises the question of how gravity affects circadian clocks. Additional studies on the earth involving simulations of the spaceflight conditions and further experiments in space [such as monitoring rhythmicity in hypogravity ($0 < g < 1$)] remain to be done before the results of the Spacelab 1 experiment can be fully understood.

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