Fungal Genetics Reports

Volume 45 Article 12

Preliminary investigation of the circadian rhythms of wild-collected Neurospora strains.

L W. Morgan
University of California

JERRY F. FELDMAN
University of California

Preliminary investigation of the circadian rhythms of wild-collected Neurospora strains.

Abstract

A medium that allows for measurement of circadian rhythms in wild-collected strains of *Neurospora* is reported. Preliminary results with *N. intermedia* strains from four different latitudes suggest natural variation in clock-affecting loci.

Preliminary investigation of the circadian rhythms of wild-collected *Neurospora* strains.

Louis W. Morgan and Jerry F. Feldman - Department of Biology, University of California, Santa Cruz, Santa Cruz, CA 95064.

A medium that allows for measurement of circadian rhythms in wild-collected strains of *Neurospora* is reported. Preliminary results with *N. intermedia* strains from four different latitudes suggest natural variation in clock-affecting loci.

Neurospora crassa is one of the best studied systems for circadian rhythms. However, almost all previous work on *Neurospora* clocks has been done in *N. crassa* laboratory strains carrying the *band* mutation because wild-type strains do not show rhythmic banding under standard conditions. This precludes easily studying the diversity of circadian rhythms in wild-collected strains. A medium containing rubidium chloride had previously been used to induce a *band*$^{+}$ strain (*csp-2*) to form conidial bands (Gall and Lysek 1981 Neurospora Newsl. **28**:13), but these authors did not use this medium for strains from nature. We have devised a variation of the rubidium chloride medium which enables period length to be measured on wild-collected strains.

Gall and Lysek used 60 mM RbCl in their medium, with fructose as a carbon source, which caused ~50% reduction in growth rate and affected viability of the *csp-2* strain at temperatures differing significantly much from 20°C. In order to reduce the amount of rubidium chloride required to induce circadian banding of conidiation we used an acetate-based medium. This acetate/casamino acids medium causes thinner bands of conidiation and a slower linear growth rate (~60%) as compared to standard glucose-arginine medium (Sargent and Kaltenborn 1972 Plant Physiol. **50:** 171-175).

Race tube medium containing 1.2% sodium acetate, 0.5% casamino acids, and 2% agar (Feldman and Hoyle 1973 Genetics **75**:605-613) was tested at 5 mM, 15 mM, and 25 mM RbCl concentrations with four wild-collected *N. intermedia* strains and an *N. crassa* strain (*band*). The race tube assay was performed at 25°C as described in Feldman and Hoyle. Standard (12 inch) race tubes allowed 6 to 7 days of growth after transfer to darkness. The results from the 15 mM RbCl medium, which gave the clearest banding, are presented in Table 1.

The linear growth rate of the *band* strain was reduced by only about 25% at 25°C by the addition of 15 mM rubidium chloride to the medium. In this assay, three of the wild *N. intermedia* strains had a circadian rhythm similar to the *N. crassa* wild-type (*band*) strain with a period length of about 22 hours. However, the P2264 strain collected in New Zealand had a long period length. This long period length may reflect naturally occurring allelic variation at a clock-affecting locus, since there are several known alleles of *N. crassa* clock genes which lengthen period length. These results show that the circadian rhythm of wild-collected *Neurospora* strains can be easily seen without the need to introduce any mutations.

These results extend possibilities for looking into natural variability in circadian behavior, within and between populations and species, in different ecological settings, and at different latitudes. For example, Pittendrigh and Takamura (1989 J. Biol. Rhythms **4:** 217-235) showed a latitudinal cline in the properties of the *Drosophila* clock using wild-collected strains. However, our experiments with wild-type strains were exploratory and

peripheral to our current research. We do not expect to have an opportunity to do further work using the rubidium medium, but we have been encouraged by Dr. David Perkins (who generously gave us the wild-collected strains) to report these preliminary results. We thank Dr. Perkins and Ken Langdon (both of Stanford University) for their enthusiasm and assistance with this study.

Table 1. Period lengths and linear growth rates of *Neurospora* strains on acetate/casamino acids medium containing 15 mM RbCl at 25°C.

Strain	n	Period length (hours)	Growth rate (mm/day)
band (no RbCl)	6	22.0 ± 0.5	24.3 ± 1.8
band	5	21.4 ± 0.7	17.7 ± 1.9
P9 (Unzen, Japan)	5	22.6 ± 0.3	33.2 ± 0.5
P277 (Singapore)	3	22.7 ± 1.1	34.2 ± 3.6
P146 (Bogor, Java)	6	22.6 ± 0.6	33.4 ± 1.9
P2264 (Ahipara, N.Z.)	3	25.7 ± 0.8	34.0 ± 0.9