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Polymorphism for pKALILO based senescence in Hawaiian populations of *Neurospora intermedia* and *Neurospora tetrasperma*

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Abstract

The natural population of *Neurospora intermedia* from Hawaii is polymorphic for the presence of the linear mitochondrial plasmid pKALILO that is associated with an infectious senescence syndrome. Although inter-specific horizontal transmission is experimentally possible, thus far pKALILO associated senescence has never been found outside *N. intermedia* in nature. Here, we demonstrate that it is not limited to the natural population of the heterothallic species *N. intermedia*, but also present in the sympatric population of its close relative, the pseudo-homothallic species *Neurospora tetrasperma*. We did a comparative analysis of the hallmarks of senescence in both species and show that: (1) Senescence is contagious in both species: the senescent state is efficiently transmitted between vegetatively compatible isolates. (2) All senescent isolates from both species contain the autonomously replicating linear mitochondrial senescence plasmid pKALILO. (3) In both species, senescent cultures contained copies of pKALILO inserted into the mitochondrial genome. Two of these inserts were characterized using semi-random two-step PCR, and were located within the large subunit mitochondrial rRNA gene. (4) However, pKALILO was less frequent in *N. tetrasperma* than in *N. intermedia*. (5) Also, the onset of senescence was significantly delayed in *N. tetrasperma*, compared to that in *N. intermedia*. We hypothesize how these differences in frequency and effect of pKALILO are connected to the respective life histories of their hosts.

Keywords: Invertron; pHANALEI-2; Virulence; Aging; Ageing

1. Introduction

Mitochondrial plasmids are ubiquitous among filamentous fungi (Griffiths, 1995). Although the vast majority of these plasmids do not appear to have any phenotypic effects on their hosts, at least some of them do (Bertrand, 2000; Griffiths, 1992). For example the linear mitochondrial plasmids pKALILO of *Neurospora intermedia* (Bertrand et al., 1985, 1986), pMARANHAR of *Neurospora crassa* (Court et al., 1991), and pAL2-1 of *Podospora anserina* (Maas et al., 2004). These plasmids, all three from well-studied species, are associated with a fungal senescence syndrome.

PKALILO naturally occurs in Hawaiian populations of the heterothallic species *N. intermedia*. Cultures containing this plasmid typically show a progressive decline in both growth rate and fertility, ultimately culminating in death. In aging cultures the plasmid inserts into the mitochondrial genome, and in those cases analyzed were found to be located within the large subunit (LSU) rRNA gene (Bertrand et al., 1985; Myers et al., 1989). For a reason that is not yet fully understood, mtDNA molecules containing an inserted plasmid are "suppressive"; i.e., they accumulate during growth, gradually replacing the wild-type molecules (Myers et al., 1989). These cultures become deficient in functional mitochondrial ribosomes and ultimately die.

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Because of its mitochondrial location, pKALILO and its concomitant senescence phenotype (Also indicated as 'Kalilo'), are usually inherited maternally. However, the plasmid can in some cases also be transmitted paternally (Yang and Griffiths, 1993a), and horizontal transfer of the free plasmid and even of the suppressive senescence phenotype, via transient hyphal fusion, has been observed (Debets et al., 1994). Transmission of the plasmid in such interactions is affected by somatic or vegetative incompatibility (Debets and Griffiths, 1998). Natural populations of *N. intermedia*, and of *Neurospora* species in general, typically show a high degree of vegetative incompatibility among isolates, which prevents or slows down the rate of infectious transmission of plasmids, mycoviruses, and similar elements. This incompatibility is based on a multi-locus self/non-self recognition system resulting in cell death when hyphae of incompatible isolates fuse. The loci involved in this process, called HET-loci, are highly polymorphic within a population (Glass et al., 2000). Despite the fact that this system constitutes a successful barrier among isolates against the infectious spread of the senescent state, it does not completely prevent cytoplasmic exchange and pKALILO can be transmitted among incompatible *N. intermedia* wild types with remarkable ease (Debets et al., 1994). Its relatively high rate of intra-specific horizontal transmission, which is in the order of 10%, can be seen as the hallmark of a virulent parasite and may partly explain the prevalence of Kalilo in the *N. intermedia* population from Kauai.

Experimental evidence has shown that Kalilo can be transmitted inter-specifically as well, via introgression and subsequent heterokaryosis (Bok et al., 1999). However, despite several large-scale surveys among *Neurospora* isolates from around the world (Arganoza et al., 1994; Yang and Griffiths, 1993b), the prototypic plasmid has only once been found outside *N. intermedia*; in an isolate of *Neurospora tetrasperma* originating from Moorea-Tahiti (He et al., 2000). The latter authors did not report whether this particular isolate showed senescence or not. Several homologues of pKALILO were found in a range of different hosts, including *Neurospora discreta*, *N. crassa*, and even in the related genus Gelasinospora (He et al., 2000; Marcinko-Kuehn et al., 1994; Yuewang et al., 1996), but all of these plasmids were substantially different from pKALILO at the DNA sequence level and, in contrast to the prototypic plasmid from *N. intermedia*, those tested neither integrated into the mitochondrial genome nor showed a convincing correlation with senescence. Thus, Kalilo itself has formally never been described in natural populations of *Neurospora* outside the Hawaiian one of *N. intermedia.* The majority of pKALILO homologue containing isolates however, has not been tested for senescence and thus the overall picture is incomplete. For this reason, we have screened the Hawaiian population of *Neurospora* for novel cases of pKALILO in other species.

In this study, we show that: (1) pKALILO and its contagious senescence phenotype is not restricted to the heterothallic species *N. intermedia*; we found a large number of cases in isolates of the pseudo-homothallic species *N. tetrasperma*. (2) Both in *N. intermedia* and in *N. tetrasperma*, senescence is associated with plasmid copies inserted into the mitochondrial genome. (3) The frequency of pKALILO, however is lower in *N. tetrasperma* than it is in *N. intermedia*. (4) Moreover, Kalilo isolates from *N. tetrasperma* are significantly longer-lived than those from *N. intermedia*. We hypothesize how these differences in frequency and effect of pKALILO are connected to the respective life histories of their hosts.

2. Materials and methods

2.1. Collection, identification, and culturing of Neurospora isolates

Soil samples were collected from Hawaii in June and July 1998. Six of them were taken from three different locations on the isle of Oahu and 36 of them were taken from 11 different locations on the isle of Kauai. Collection sites were 1–10 km apart and within sites, samples were taken several meters apart. Soil was pasteurized to obtain *Neurospora* isolates (Perkins and Turner, 1988): Samples were incubated for 30 min at 60 °C in modified Westergaard's Medium (WM), pH 6.3. Chloramphenicol (0.3 g/L medium) was used to suppress bacterial growth and 1 ml of 10% (v/v) 2-furylmethanol (furfural) to improve ascospore germination. Following pasteurization, soil was incubated at 25 °C for up to two weeks. As soon as they appeared, *Neurospora* colonies were isolated and subcultured on Vogel's Minimal Medium (VMM), pH 5.8 (Davis and de Serres, 1970). From each isolate, conidia were stored in 87% glycerol at −80 °C or as silica-gel stocks as previously described (Perkins and Turner, 1988).

For species identification, all isolates were crossed with known reference isolates as described by Perkins and Turner (1988). *N. intermedia* isolates FGSC #3416 (mating type A) and FGSC #3417 (mating type a) and *N. crassa* isolates FGSC #5798 (mating type A), FGSC 4317 (mating type A) and FGSC #4347 (mating type a) were used as reference. Self-fertile isolates producing four-spored asci did not show interfertility with any of these and were classified as pseudo-homothallic *N. tetrasperma* isolates.

Senescence was phenotypically demonstrated using serial subculturing as described by Griffiths and Bertrand (1984). Life span was expressed either in days or in number of subcultures. Standard working protocol for *Neurospora* was as given by Davis and de Serres (1970).