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Evidence for the absence of meiotic silencing by unpaired DNA in *Neurospora tetrasperma*

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Abstract

Meiotic silencing by unpaired DNA is a posttranscriptional gene silencing process in *Neurospora crassa*. Any gene without a homolog in the same chromosomal position during meiotic prophase generates a sequence-specific signal that prevents expression of all copies of that gene, but only during meiosis. Meiotic silencing is epigenetic and involves components of a meiosis-specific RNA silencing machinery. Although *N. tetrasperma* is closely related to *N. crassa*, its sexual biology is significantly different. *N. tetrasperma* was used here to evaluate both the generality of meiotic silencing within the genus and its possible evolutionary significance. A reporter gene for meiotic silencing, a histone H1-GFP fusion construct, was introgressed from *N. crassa* into various chromosome locations in *N. tetrasperma*. Whereas we did not observe meiotic silencing in four out of five introgression series, we obtained inconclusive results in the fifth series. Thus, we propose that meiotic silencing in *N. tetrasperma* is either absent or is substantially reduced when compared to *N. crassa*, possibly because the *sad-1* gene (RNA-directed RNA polymerase, RdRP) is naturally unsynapsed (although ''paired'') and self-silenced during meiosis by structural differences between *N. tetrasperma* mating-type chromosomes. In *N. crassa*, wild-type *sad-1* function is essential for meiotic silencing. Many point mutations in or deletion of *sad-1* result in self-silencing of RdRP, and consequently suppression of meiotic silencing in heterozygous asci. The apparent absence or reduced meiotic silencing in *N. tetrasperma* raises the possibility that this form of silencing is not necessarily a major genome defense mechanism or responsible for reproductive isolation among the species of the genus *Neurospora*.

Keywords: *Neurospora*; Pseudohomothallism; Mating-type chromosome; Recombination block; Sex chromosome

1. Introduction

Meiotic silencing by unpaired DNA is a posttranscriptional gene silencing (PTGS) process, first discovered in *Neurospora crassa* (Aramayo and Metzenberg, 1996; Shiu et al., 2001). During meiotic prophase, unpaired genes (i.e., without a homolog in the same chromosomal position) generate a sequence-specific signal that prevents expression of all copies of that gene. Evidence to date suggests that meiotic silencing is related to RNA interference (RNAi), similar to ''quelling'' in fungi and ''co-suppression'' in plants, but restricted to the meiotic phase of the life cycle (Shiu et al., 2006). Meiotic silencing has been postulated to provide an important defense mechanism for genome integrity as it may prevent expression and transposition of invasive DNA elements, and to serve as a key genetic component in the reproductive isolation among species of *Neurospora*. *N. tetrasperma* is a closely related sister species of *N. crassa*, but with a significantly different sexual biology. In this study, we have used *N. tetrasperma* to evaluate both the generality of

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meiotic silencing within the genus and its possible evolutionary significance.

Sexual reproduction in *N. crassa* requires crossing of two haploid individuals of opposite mating types, *mat A* and *mat a*, at the single-mating-type locus on linkage group I (LGI). In this heterothallic species, each ascus (meiocyte) produces eight individual haploid ascospore progeny, following meiosis and a postmeiotic mitosis (Raju, 1980). In contrast, *N. tetrasperma* packages the eight haploid nuclei as four non-sister pairs (*mat A* + *mat a*) into each of four ascospores (Fig. 1) (Dodge, 1927; Raju, 1992). Each progeny ascospore, thus contains both mating-type nuclei, and is competent to complete sexual reproduction by selfing, without having to outcross. This sexual strategy (pseudohomothallism), although unique to and relatively rare in fungi, has evolved independently in disparate fungal lineages across the kingdom (Raju and Perkins, 1994).

The ascus programming that is necessary to consistently produce self-fertile progeny in *N. tetrasperma* requires specific chromosome and nuclear behavior to assure that mating types (*mat A* and *mat a*) segregate at the first division of meiosis. This is accomplished by strict linkage of the mating-type locus to the centromere combined with overlapping spindles at the second and third divisions, and precise positioning of non-sister pairs of nuclei for inclusion into each of the four ascospores (Raju, 1992). Any crossovers in the interval between mating type and centromere would produce self-sterile (i.e., same mating-type) progeny in one-half of asci. A large genetically controlled recombination block on the mating-type chromosome provides the necessary linkage of *mat* and centromere. Correlated to this recombination block is a large unsynapsed region in the mating-type chromosome that is cytologically visible during pachytene (Fig. 2) (Gallegos et al., 2000; Jacobson, 2005); the lack of synapsis is indicative of physical non-pairing of loci at the DNA level, although DNA pairing has not been shown to be a requirement for meiotic silencing. The remaining six chromosomes of *N. tetrasperma* show normal synapsis and recombination; these have been referred to as autosomes.

The recombination block and unsynapsed region encompasses ~60% of the mating-type chromosome, which is ~10% of the total genome, and likely contains genes essential to meiosis and sexual reproduction. Therefore, a

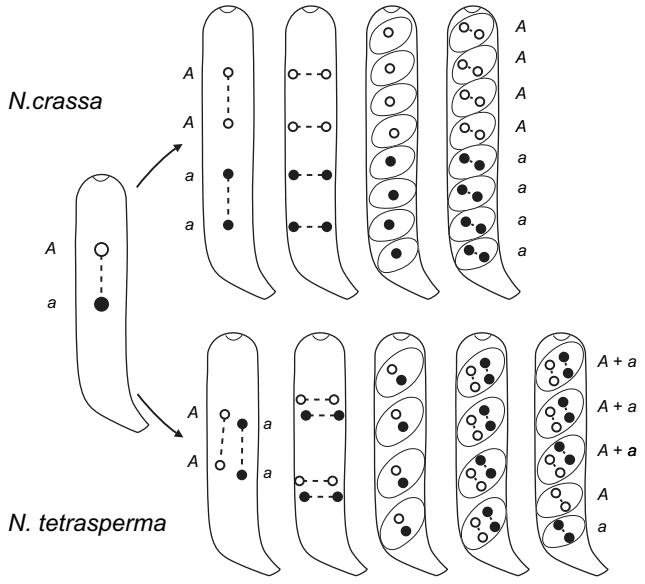

Fig. 1. Diagram of ascus development in *Neurospora crassa* and *N. tetrasperma*. *N. crassa* is eight-spored and heterothallic, whereas *N. tetrasperma* is four-spored and pseudohomothallic. In both species, mating-types segregate at the first division of meiosis, but the spindles at the second and third divisions overlap in *N. tetrasperma* such that each of the four ascospores encloses two nuclei of opposite mating type. Thus, single-ascospore cultures are normally heterokaryotic and self-fertile. However, abnormal nuclear behavior in a few exceptional asci results in more than four ascospores, where one or more, large, heterokaryotic ascospores are replaced by pairs of small homokaryotic ascospores. (Adapted from Raju and Perkins, 1994.)

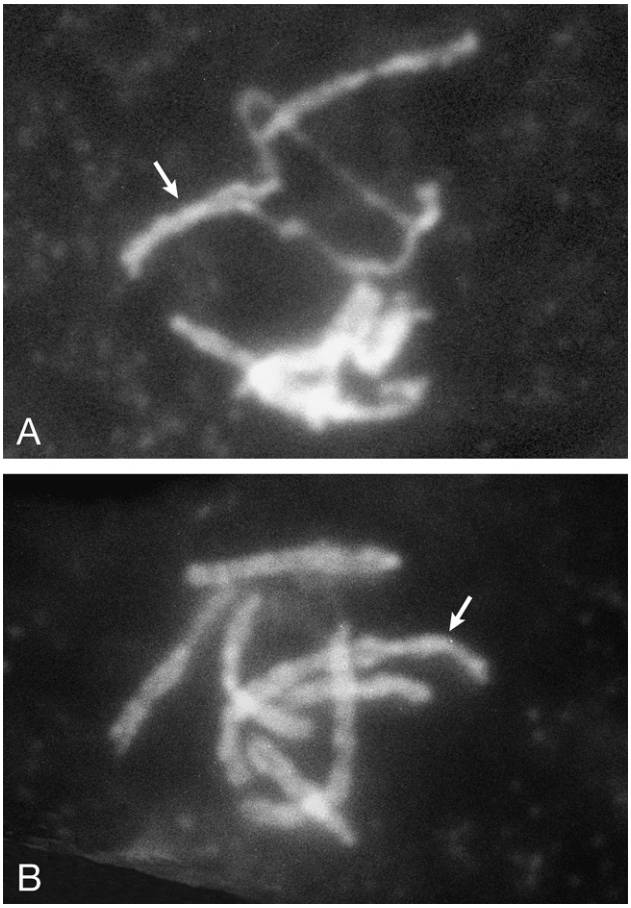

Fig. 2. Pachytene chromosomes of *N. tetrasperma* and *N. crassa* (acriflavine staining). (A) Selfing of *N. tetrasperma* (P535 *mat A* × P535 *mat a*). Note the long unpaired region on the longest chromosome (see arrow). This unpaired region correlates with the recombination block in the mating-type chromosome. (B) Cross of *N. crassa* × *N. intermedia*. Note complete pairing along the entire length of the longest chromosome (mating-type chromosome, see arrow). The karyotypes of these closely related species are similar and show normal pairing in both conspecific and interspecific crosses. The chromosomes show better spreading in certain interspecific crosses. (Fig. 2A from Gallegos et al., 2000, and Fig. 2B from Raju, 1986.)