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Detection of *Sad* genes in various species of *Neurospora*

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Neurospora crassa is a haploid fungus that reproduces asexually during vegetative growth. However, in a nitrogen deficient environment, the two mating-type cells, *A* and *a*, can fuse together and enter the sexual cycle. During this transient diploid stage, a post-transcriptional gene silencing (PTGS) mechanism silences the expression of any unpaired gene. This mechanism is called meiotic silencing by unpaired DNA (MSUD) (Shiu, *et al.*, 2001; Cell 107: 905-916). MSUD occurs when a gene is not paired with a homologue during meiosis. The unpaired DNA segment generates a sequence-specific signal, causing any paired or unpaired copies of that gene to be silenced. Two required genes for meiotic silencing have been identified; *sad-1* encodes an RNA-directed RNA polymerase while *sad-2* encodes a novel protein. Interspecific crosses between *N. crassa* and other related species are known to be infertile. This infertility may be due rearrangements caused by inversion and/or translocation. These could disrupt the pairings of genes needed for meiosis and ascus development, causing these genes to be silenced, resulting in sterility. This hypothesis is validated by observing that interspecific crosses within the genus *Neurospora* become much more fertile when the *N. crassa* parent contains a *Sad-1* mutation. If MSUD is in other *Neurospora* species, it may represent another mechanism for speciation. This research project focuses to determine whether *sad-1* and *sad-2* are conserved in related species of *Neurospora*. Homologues of the sad genes were amplified in several fungal isolates by PCR (Polymerase Chain Reaction), using primers designed for those sequences. We have discovered that *sad-1* and *sad-2* can be found in several different *Neurospora* species; *sad-1* is present in *N. sitophila, N. tetrasperma, N. dodgei, N. galapagosensis*, and *N. africana*, while *sad-2* is found *in N. sitophila, N. tetrasperma*, and *N. intermedia*. The presence of *sad* genes in these species suggests MSUD may contribute to their reproductive isolation.