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Map-based cloning and characterization of meiotic drive resistance

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Recent sequencing of the Neurospora crassa genome by The Broad Institute, in collaboration with the Neurospora research community, has given considerable insight into the understanding of Neurospora's DNA as well as its molecular structure and function. With this sequencing information, we are able to more easily characterize important loci within the N. crassa genome. One such locus, Spore killer-3 (Sk-3), is of particular interest to our laboratory. Sk-3 is considered a meiotic drive element because it segregates in excess of its Mendelian proportion during mating. In other words, instead of half the offspring receiving the Sk-3 allele, and the other half receiving the wild type allele, the Sk-3 allele is passed to nearly all of the offspring. Meiotic drive elements are often categorized as "selfish genetic elements." The reason for our interest in selfish genetic elements is that many of them cause diseases in humans. Model systems, like N. crassa, may reveal clues to their functions in higher eukaryotes. In this project, we are examining the Spore killer resistance gene (r(Sk-3)) in an attempt to gain further insight into the purpose and function of Sk-3 and ultimately of meiotic drive elements. The N. crassa genome sequence annotation has putatively placed the r(Sk-3) gene to the left of the centromere on chromosome III of the N. crassa genome. Using closely linked loci, some of which contain the hph (resistance to hygromycin) marker, we have utilized a standard three-point cross strategy to refine the known location of r(Sk-3). Mapping with additional markers is currently in progress to help narrow the r(Sk-3) locus to a ~20kb region. Genes from this region will be individually cloned and introduced into a Sk-3 susceptible host. Resistance to killing in the transformants will be used to identify the r(Sk-3) activity.