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Analysis of the mtDNA insertion site on chromosome 9L in maize inbreds using fluorescence in situ hybridization

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Almost all eukaryotic nuclear genomes show evidence of organellar DNA insertions originating from mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA). While the precise mechanisms of incorporation remain unknown, the phenomenon is frequent and ongoing in many species. In *Zea mays*, mtDNA insertions differ among inbred lines. A very large mtDNA insertion is found near the centromere of the long arm of chromosome 9 in the B73 inbred. This insertion contains the majority of the mitochondrial genome, while a similarly positioned insertion in the Mo17 inbred line is much smaller. We used recombinant inbred lines from the intermated B73 x Mo17 (IBM) population to determine if the insertions are indeed at the same position. We selected lines with recombination in this region of chromosome 9L. Using two mtDNA probes present in the insertions in both B73 and Mo17, we applied a chromosome painting technique called fluorescence in situ hybridization (FISH) to root-tip metaphase chromosomes and looked for the presence of the mtDNA site on chromosome 9L in the selected IBM lines. If the mtDNA insertion sites in B73 and Mo17 are at different locations, then at least one of the recombinant IBM lines should not display a mtDNA insertion at the chromosome 9 location. However, all of the recombinant IBM lines examined displayed the mtDNA insertion site on chromosome 9L. This indicates that the Mo17 and B73 insertions likely occupy the same region on the chromosome. Furthermore, this suggests that the large mtDNA insertion occurred recently in B73 at a pre-existing site present in both B73 and Mo17.