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Identification of chloroplast DNA insertions in B73 nuclear chromosomes

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It is known that DNA from mitochondria and chloroplasts migrates to the nucleus and incorporates into the nuclear genome. It is unknown how often this transfer and integration process occurs or if specific sites in the chromosomes are preferred. We have evidence that chloroplast DNA has integrated into multiple sites within chromosomes in the B73 inbred line of maize. This has been shown with a procedure called FISH, Fluorescence in situ Hybridization, in which fluorescent probes created from DNA hybridize with chromosomes in places where there is a match. Probes for this project were generated from chloroplast DNA. The chloroplasts were separated by centrifugation and lysed to obtain the DNA. The chloroplast genome was subdivided into 15 regions. Pieces of chloroplast DNA corresponding to 14 of the 15 regions were amplified using PCR (the polymerase chain reaction). The amplified DNA was purified and labeled with fluorescent dyes to create probes. These probes were subsequently hybridized to metaphase chromosome spreads on slides. The probes recognized and bound to DNA sequences in the chromosomes. With the completion of the chloroplast DNA mapping, the B73 inbred maize line will have a complete diagram of the locations of major chloroplast DNA insertions.