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A reference genetic map of *Citrus clementina*; citrus evolution inferences from comparative mapping

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The availability of a saturated genetic map of clementine was identified by the ICGC as an essential prerequisite to assist the assembly of the reference whole genome sequence based on a 'Clemenules' clementine derived haploid. The primary goals of the present study were to establish a clementine reference map, and to perform comparative mapping with pummelo and sweet orange. Five parental genetic maps were established with SNPs, SSRs and InDels. A medium density reference map (961 markers for 1084.1 cM) of clementine was established and used by the ICGC to facilitate the chromosome assembly of the haploid genome sequence. Comparative mapping with pummelo and sweet orange revealed that the linear order of markers was highly conserved. The map should allow reasonable inferences of most citrus genomes by mapping next-generation sequencing data against the haploid reference genome sequence. Significant differences in map size were observed between species, suggesting variations in the recombination rates. Skewed segregations were frequent and higher in the male than female clementine. The mapping data confirmed that clementine arose from hybridization between 'Mediterranean' mandarin and sweet orange and identified nine recombination break points for the sweet orange gamete that contributed to the clementine genome. Moreover it appears that the genome of the haploid clementine used to establish the citrus reference genome sequence has been inherited primarily from the 'Mediterranean' mandarin.

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The chromosomes of citrus: from a unifying nomenclature to the evolution of karyotypes

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Most citrus species have a small genome compartmentalized in nine chromosome pairs ($2n=18$). Their small chromosomes were differentiated in types based on the distribution of heterochromatic blocks when the fluorochromes CMA and DAPI were applied. Several cultivars could then be distinguished and hybrids identified. Only recently, FISH (fluorescent *in situ* hybridization) of ribosomal DNA genes (5S and 45S rDNA) and BACs (bacterial artificial chromosomes) from *Poncirus trifoliata*, in combination with the CMA/DAPI staining, were used to establish chromosome-specific markers. We have used these markers to identify each chromosome pair and build cytogenetic maps of *P. trifoliata* cv. 'Flying Dragon', *Citrus reticulata* cv. 'Cravo', *Citrus maxima* cv. 'Pink' and *Citrus medica* var. 'Ethrog'. Because the same markers were applied to these species, a unifying chromosome nomenclature could be proposed. No breakdown of synteny was detected, but most homeologous chromosomes varied in type, indicating differential expansion and contraction of heterochromatin. While chromosomes 1, 4 and 8 were conserved, chromosome 3 was especially variable, suggesting additional structural rearrangements such as inversions. Those markers were also useful to identify the parental chromosomes 2, 3 and 6 in the hybrid *Citrus sinensis* cv. 'Valencia', corroborating *C. maxima* and *C. reticulata* as its putative ancestors. The present addition of anchored mandarin BACs to the cytogenetic map will integrate it to the citrus genome sequence.

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