

Unraveling the novel sex determination genotype with “ZY” and a distinctive 2.15-2.95 Mb inversion among poplar species through haplotype-resolved genome assembly and comparative genomics analysis

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Abstract: *Populus tomentosa*, an indigenous tree species, is widely distributed and cultivated over 1,000,000 km² in China, contributing significantly to forest production, ecological conservation, and urban-rural greening. Although a reference genome is available for *P. tomentosa*, the intricate interspecific hybrid origins, chromosome structural variations and sex determination mechanisms remain confusion and unclear due to its broad and even overlapping geographical distribution, extensive morphological variations, and cross infiltration among white poplar species. We conducted a haplotype-resolved *de novo* assembly of *P. tomentosa* elite individual GM107, which comprises subgenomes a and b with a total genome size of 714.9 Mb. We then analyzed the formation of hybrid species and the phylogenetic evolution and sex differentiation across the entire genus. Phylogenomic analyses suggested that GM107 likely originated from a hybridization event between *P. alba* (♀) and *P. davidiana* (♂) approximately 3.8 Mya. A total of 1,551 chromosome structural variations were identified between the two subgenomes. More noteworthy, a distinctive inversion structure spanning 2.15-2.95 Mb was unveiled among *Populus*, *Tacamahaca*, *Turaga*, *Aigeiros* poplar species, and *Salix*, highlighting a unique evolutionary feature. Intriguingly, a novel sex genotype of the ZY type, which represents a crossover between XY and ZW systems, was identified and confirmed through both natural and artificial hybrids populations. These novel insights offer significant theoretical value for the study of the species' evolutionary origins and serve as a valuable resource for ecological genetics and forest biotechnology.

KEYWORDS: *Populus tomentosa*, haplotype-resolved genome assembly, chromosome structural variations, parental origin, sex determination, interspecific hybrid.