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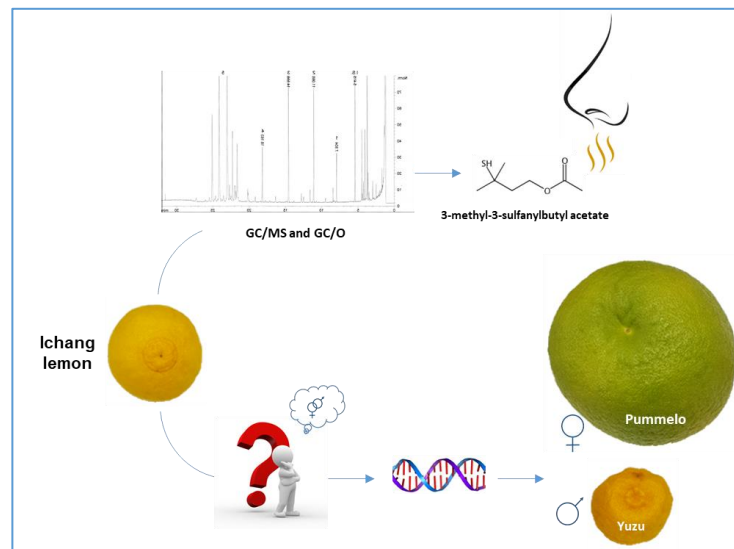
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The essential oils in citrus taxonomy: strengths and limitations of chemotaxonomy versus genetic phylogeny

François Luro, Clémentine Baccati, Patrick Ollitrault & Félix Tomi



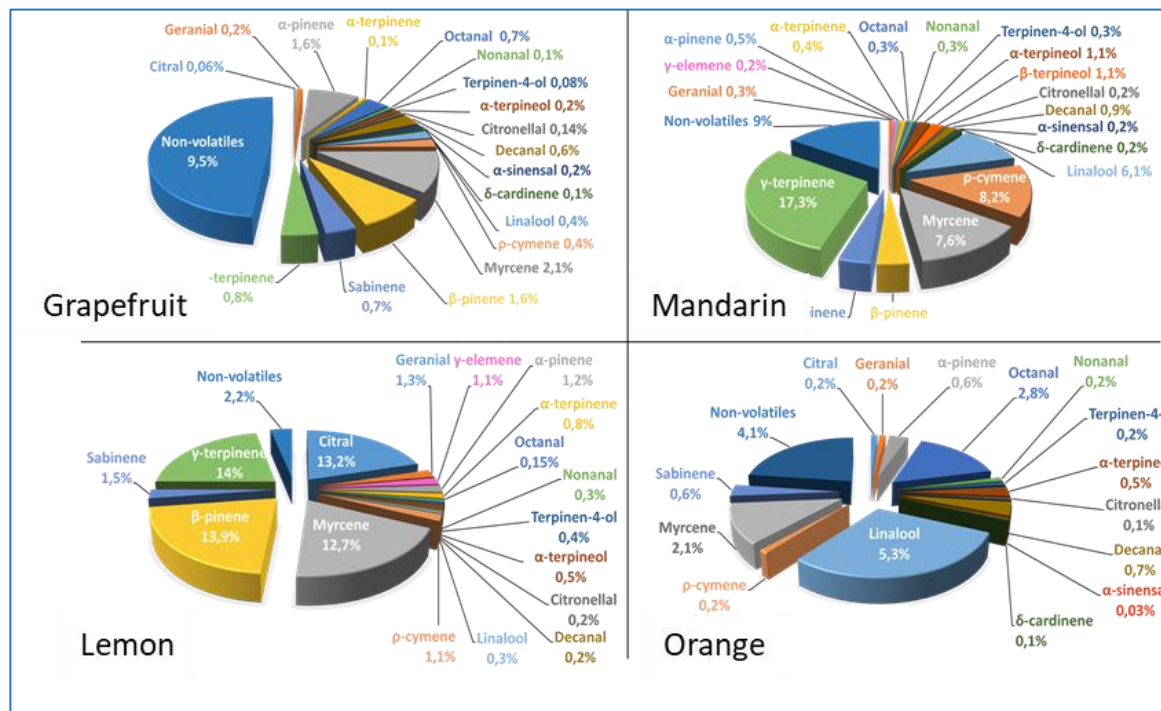
Francois.luro@inrae.fr



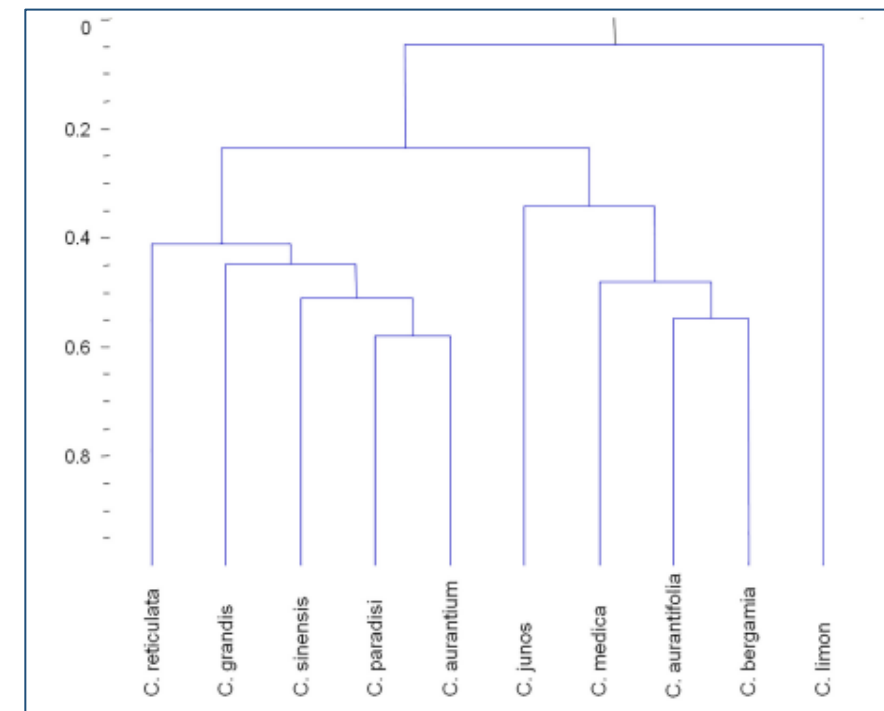
The composition of essential oil in citrus taxonomy

Each *Citrus* species has a unique organoleptic signature : mixture of major constituents (monoterpenes and sesquiterpenes and many oxygenated derivatives) and sometimes to the presence of minor components (*Dugo et al. 2011*)

Many studies of citrus classification were displayed with essential oils



Mahato et al., 2017

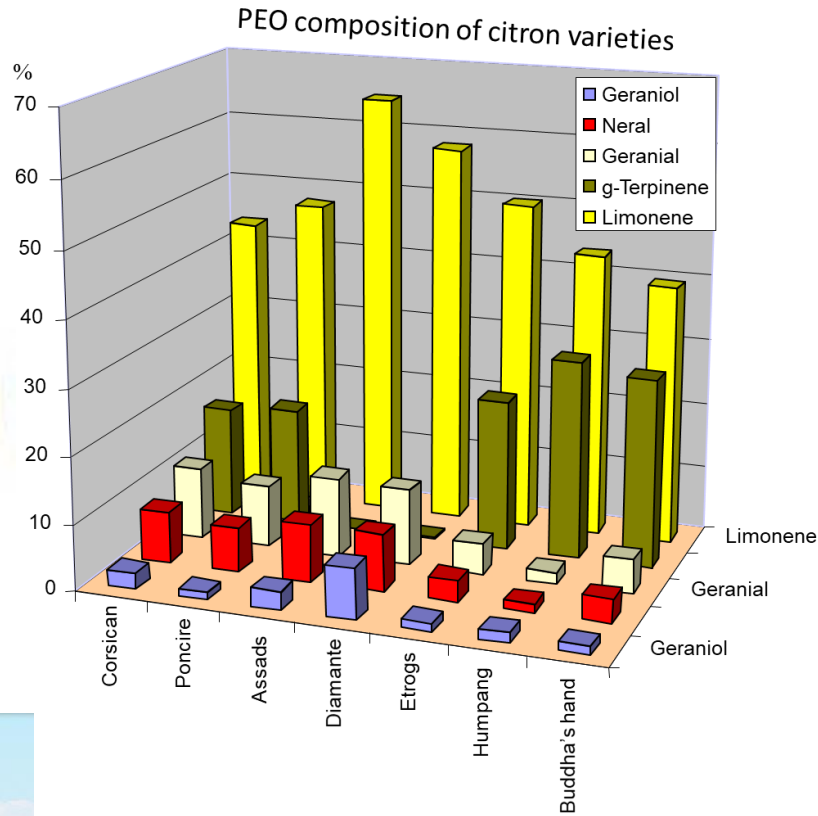


Gonzales-Mas et al. 2019

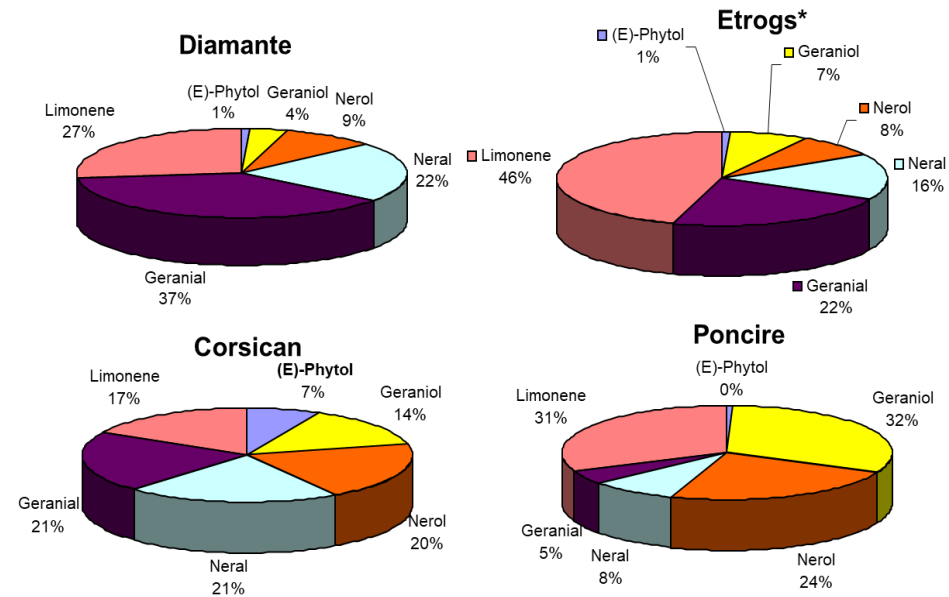
The use of the composition of essential oil in citrus taxonomy

Useful to detect intraspecific variations in a taxa and to highlight a specific chemotype

Luro et al. 2012



Major compounds of LEO in citrons



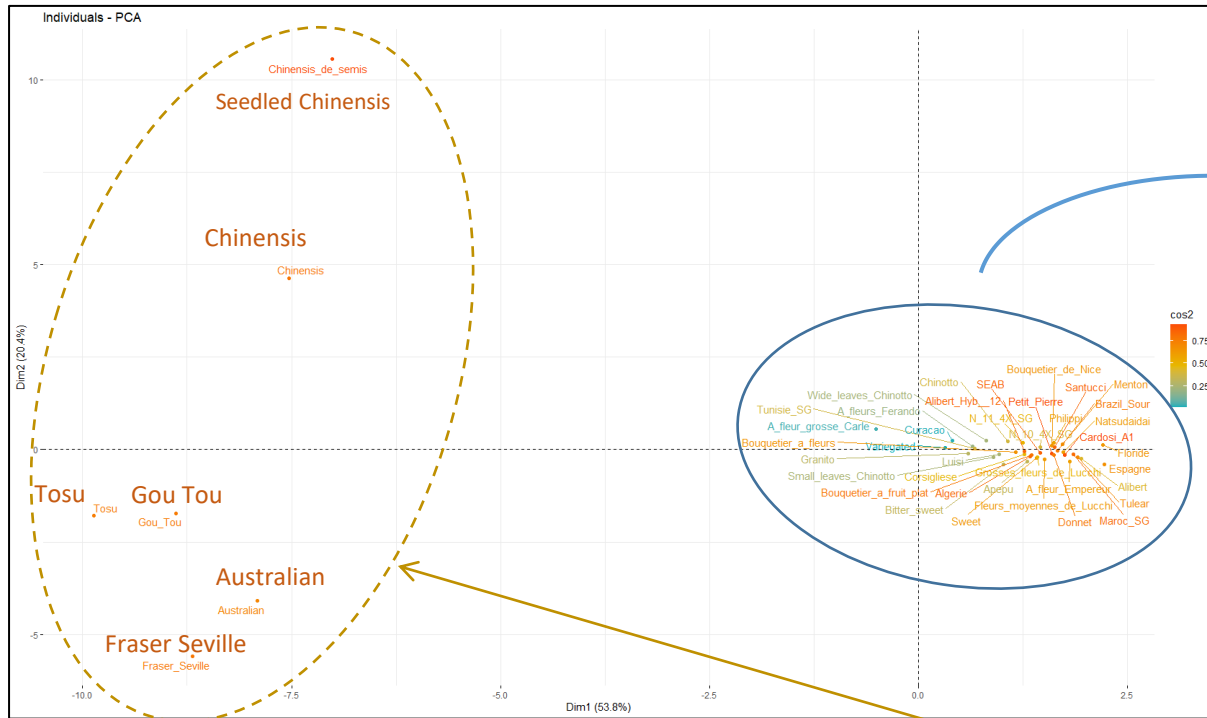
The aromatic profiles (PEO and LEO) of Corsican citron are close to Poncire cultivar in agreement with their phylogenetic relation suggested by molecular markers but also very distinct from all other cultivars

The use of the composition of essential oil in citrus taxonomy

Useful to detect non true type aromatic profile in horticultural group

Ferrer et al. 2021

Sour orange EO diversity



C. aurantium



C. neoaurantium



The sour orange chemotype is quite unvariable
 The observed variation was related to a different genetic origin of some accessions than *C. aurantium* (attested by molecular markers)

What is the contribution of aromatic EO composition in resolving genetic origin or classification when the genotype is unknown or when it is derived from several interspecific crosses?

Two examples that demonstrate the limits of chemotaxonomy in unconventional situations



Scientia Horticulturae

Volume 299, 1 June 2022, 111018



Phylogenetic and taxonomic status of *Citrus halimii* B.C. Stone determined by genotyping complemented by chemical analysis of leaf and fruit rind essential oils

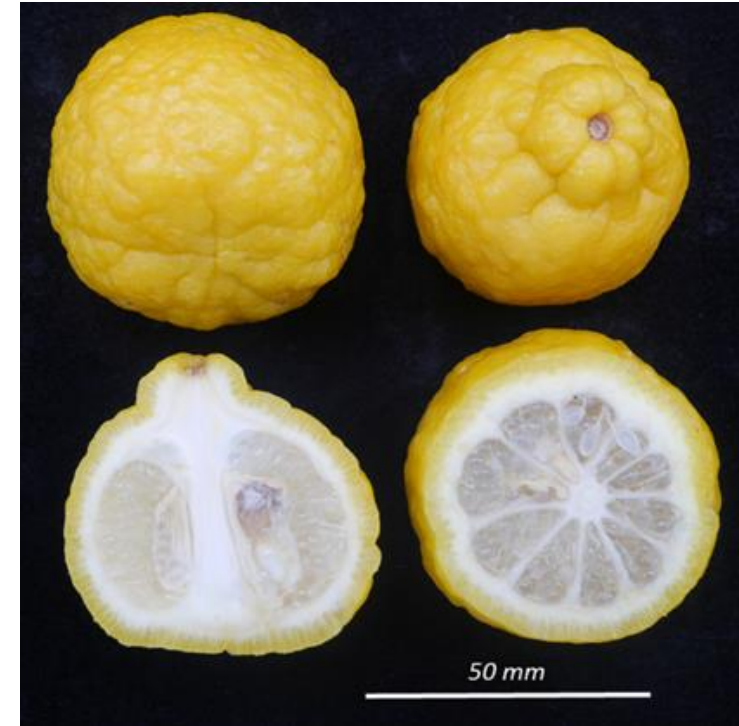
François Luro ^a, Clémentine Baccati ^b, Mathieu Paoli ^b, Elodie Marchi ^a, Gilles Costantino ^a, Marc Gibernau ^b, Patrick Ollitrault ^{a, c}, Félix Tomi ^b

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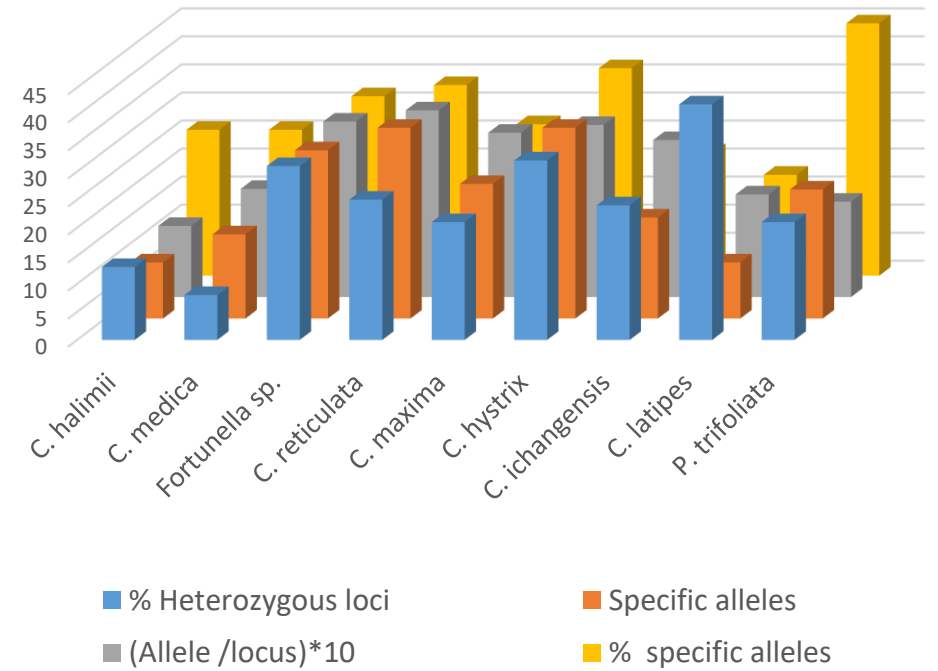
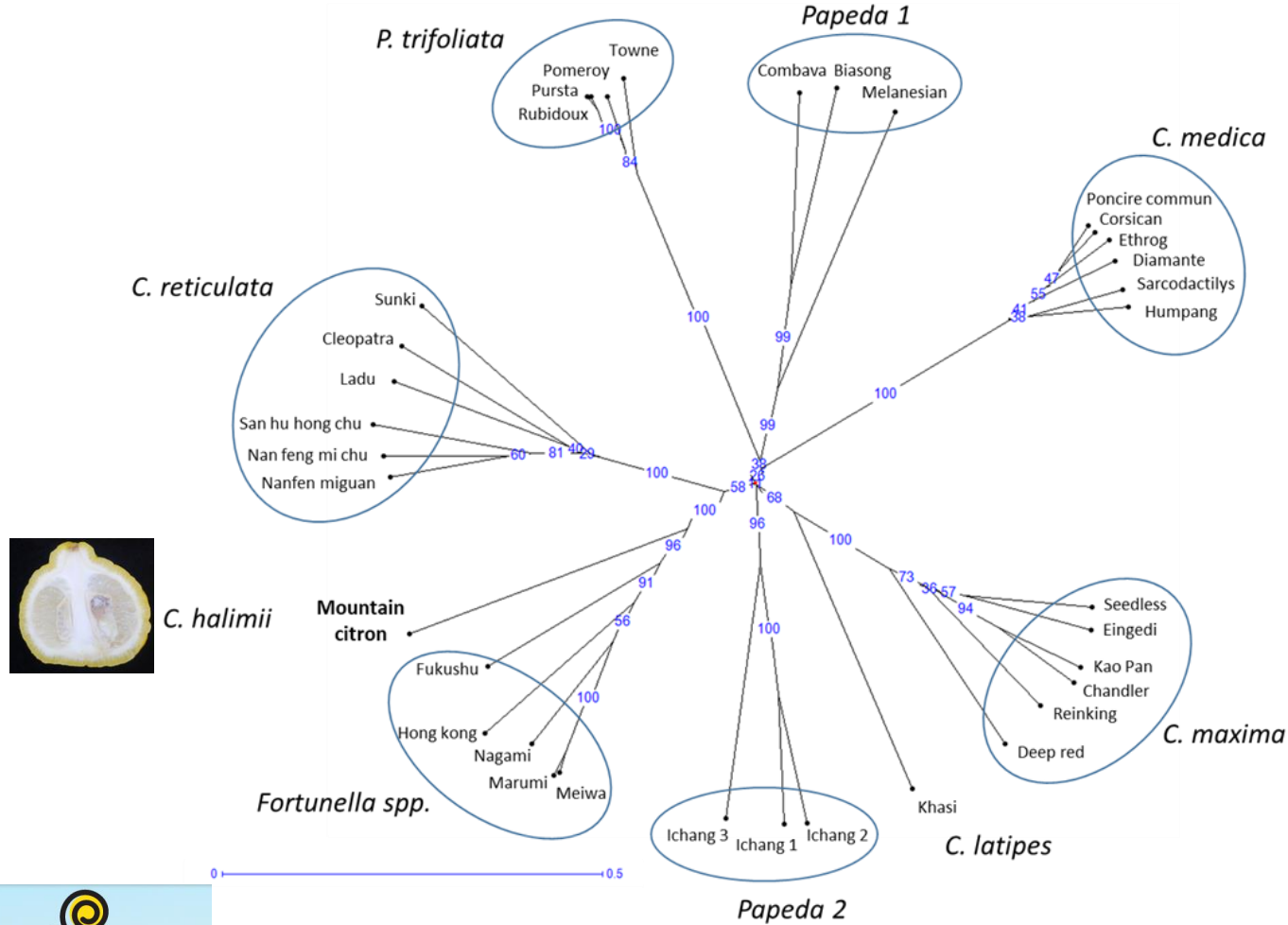
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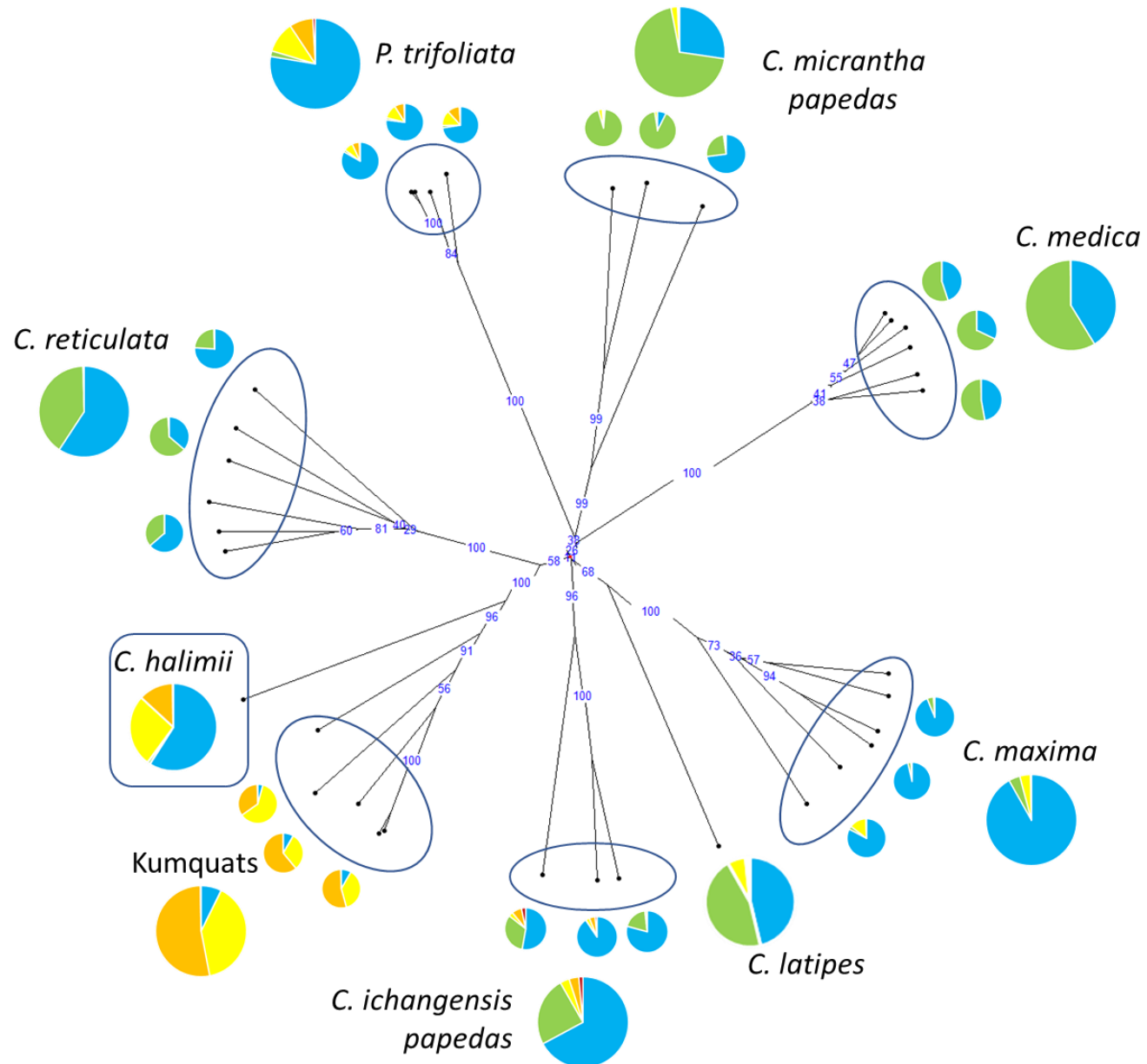


Genetic relationship of *C. halimii* with the basic taxa of Asian citrus

30 SSRs & InDels



Correspondences between genetic diversity and chemical profiles

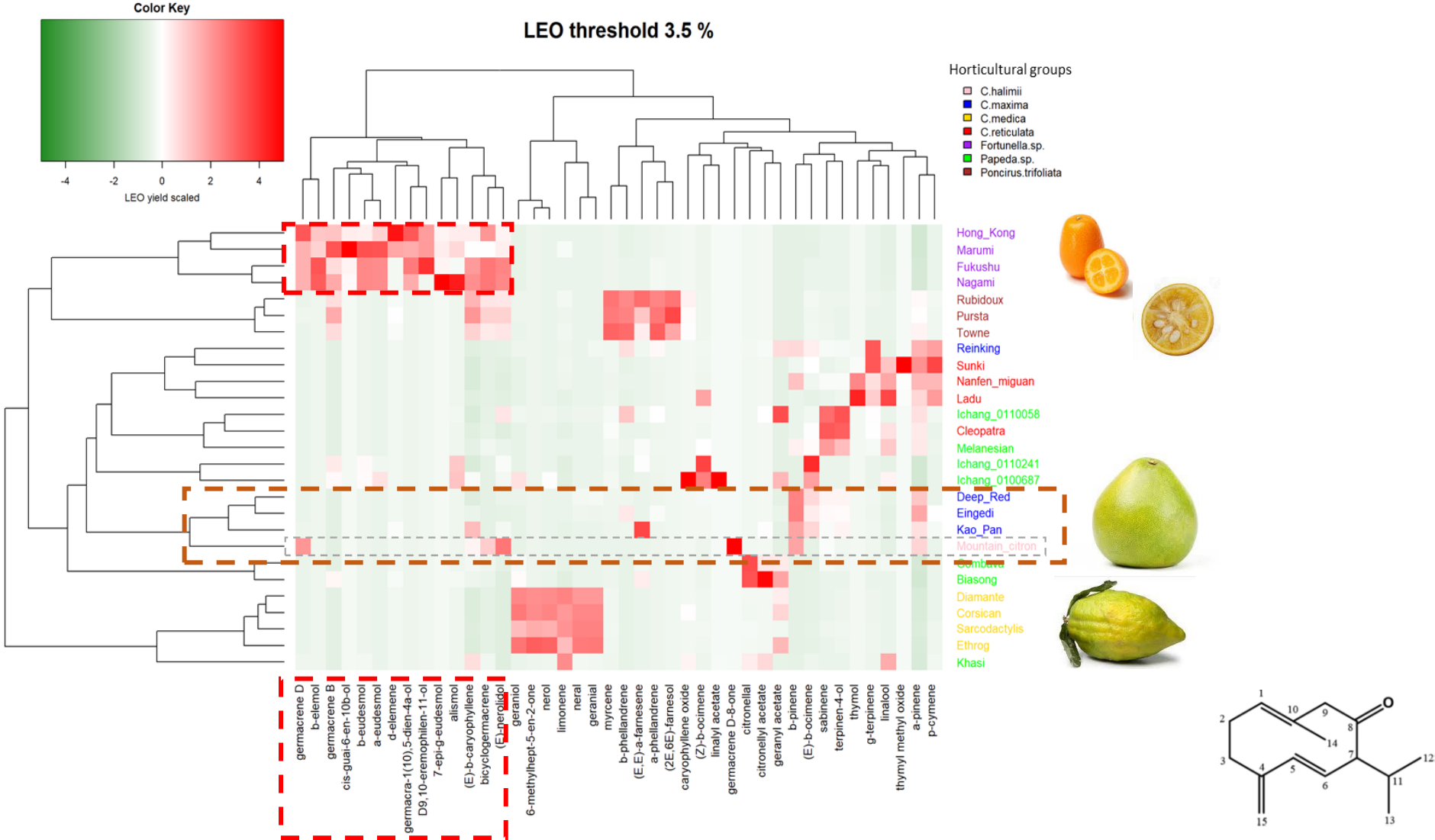


Families of aromatic compounds

- MH: Hydrocarbon monoterpenes
- OM: Oxygenated monoterpenes
- HS: Hydrocarbon sesquiterpenes
- OS: Oxygenated sesquiterpenes
- OD: Oxygenated diterpenes

Correspondences between genetic diversity and chemical profiles

According to aromatic compounds of leaf essential oils



Characterization of Odor-Active Compounds of Ichang Lemon (*Citrus wilsonii* Tan.) and Identification of Its Genetic Interspecific Origin by DNA Genotyping

Benoit Demarcq,* Margaux Cavailles, Laetitia Lambert, Christine Schippa, Patrick Ollitrault, and Francois Luro



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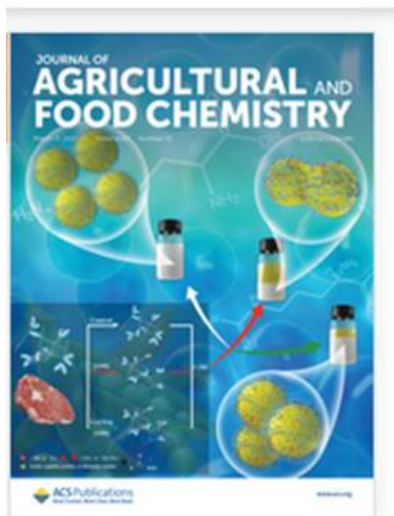


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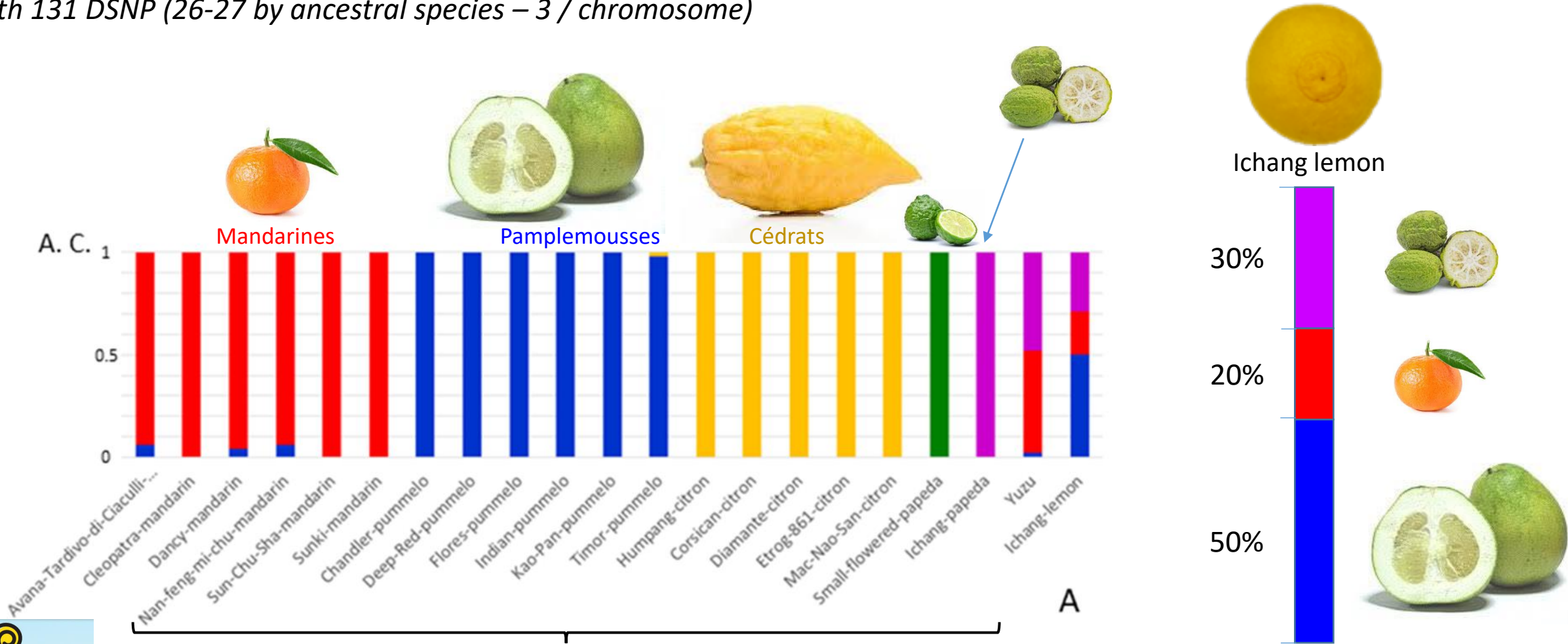
<https://dx.doi.org/10.1021/acs.jafc.0c07894>
J. Agric. Food Chem. 2021, 69, 3175–3188



Journal of Agricultural
and Food Chemistry

Ichang lemon genomic composition according to ancestral species

With 131 DSNP (26-27 by ancestral species – 3 / chromosome)



Verification of the specificity of the DSNPs for each of the ancestral species

Homozygosity/heterozygosity of DSNPs

Dark = homozygous
Light = heterozygous

Genotypic frequencies



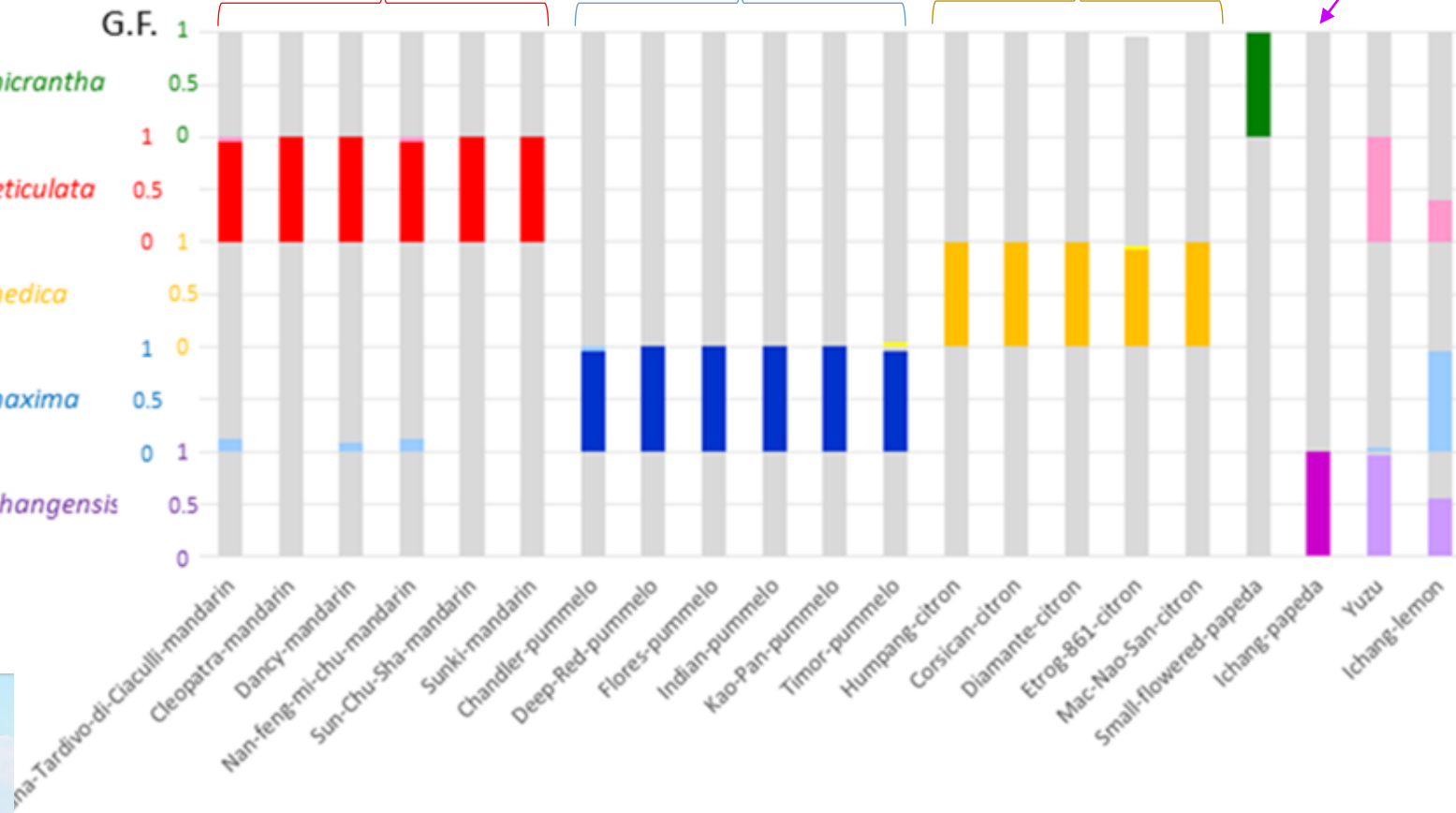
Ichang lemon

All DSNPs are heterozygous
=> direct hybrid pummelo x ?



Yuzu

All DSNPs are heterozygous
=> direct hybrid ichang papeda x mandarin



Only Yuzu has a common allele with Ichang lemon in all 27 SSR loci (100%)

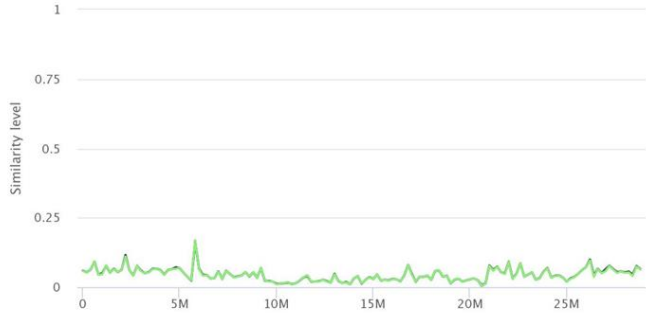
Ichang lemon genome similarity with ancestral species

Chromosome 1

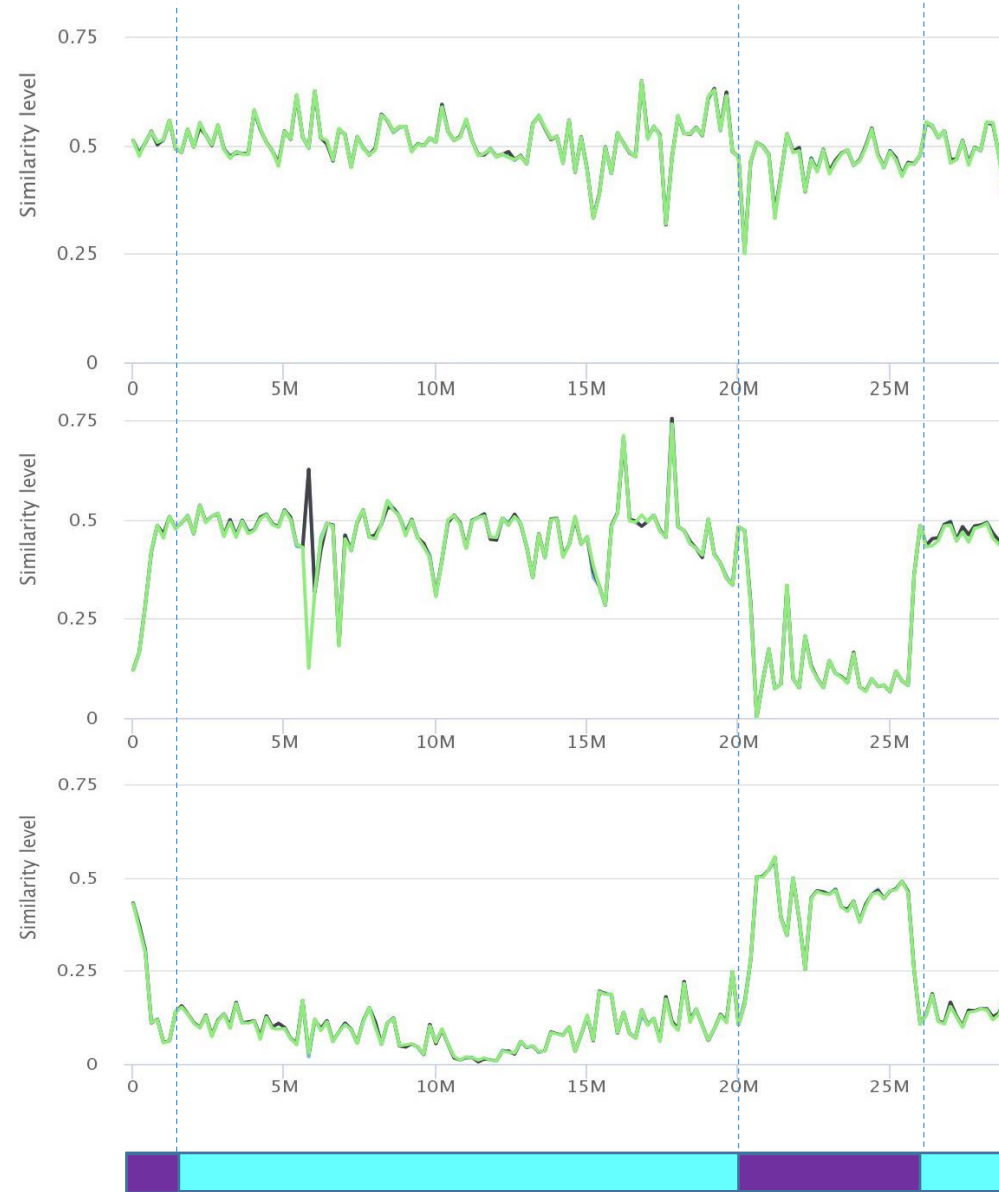
Citron

Similarity with barycentre (1)

Sliding window: 200kb



No similarity with citrons and 'micrantha' group



Pummelo



C. ichangensis

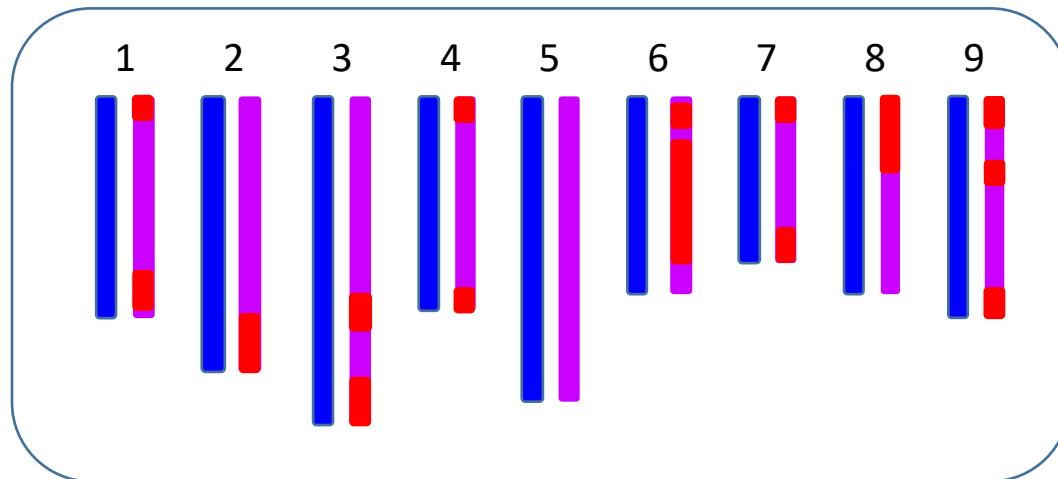
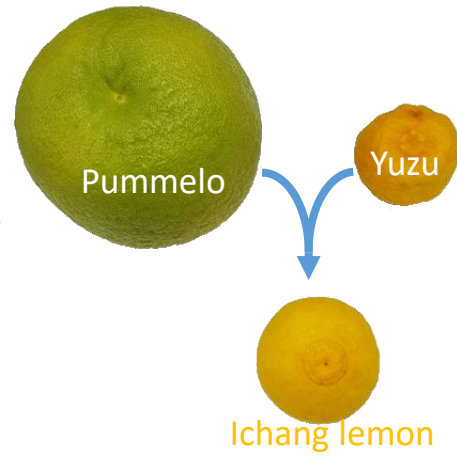


Mandarins



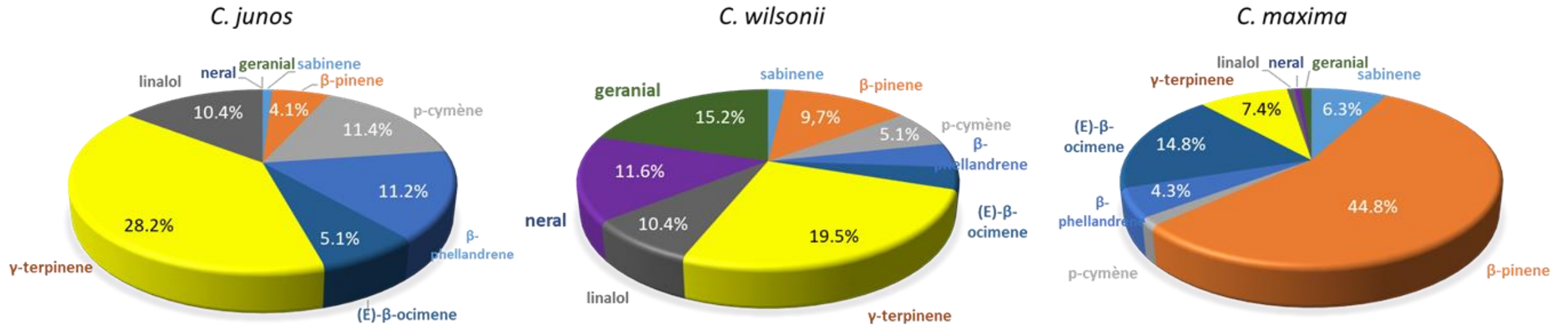
- Mandarin/Pummelo
- Pummelo/Ichangensis

Ichang lemon genome admixture



50% *C. maxima*
36.5% *C. ichangensis*
13.5% *C. reticulata*

The composition of leaf essential oils Ichang lemon and its parents



Conclusion

- ✓ The effectiveness of chemotaxonomy is often limited to horticultural groups whose variation is based solely on mutation
- ✓ The interest of essential oil analysis is especially evident in the identification of chemotypes or the discovery of particular aromatic profiles within a species/ exploitable by the aroma industry
- ✓ The EO does not always make it possible to highlight close or distant genetic relationships / genetic regulation of biosynthesis pathways is complex and dependent of environment, allelic diversity is very high due to the allopatric evolution of species.
- ✓ Some genome changes occurred in ancestors of current species and inherited by their descendants. The sequencing of the genome of many varieties allows to detect them and can serve as markers of species and then for citrus phylogeny and taxonomy