

Gourds and Tendrils of Cucurbitaceae: How Their Shape Diversity, Molecular and Morphological Novelty Evolved via Whole-Genome Duplications

Whole-genome duplications (WGDs) are regarded as important drivers of macroevolution in plants (Clark and Donoghue, 2018). However, there are few studies that test a connection between WGDs and evolutionary novelty. The proper study of evolutionary patterns related to WGDs needs a well-resolved phylogeny, accurate dates of the WGD events, and historical reconstructions of the evolutionary novelties (Clark and Donoghue, 2018). The advent of exhaustive phylogenomic studies is becoming a promising way to solve these issues, as whole-genome data can help clarify obscure phylogenetic relationships, evaluate patterns associated with WGDs, and track down the evolution of life-history traits that shaped plant lineages (Bellot et al., 2020).

The cucurbits (Cucurbitaceae) are a monophyletic angiosperm family with ca. 1000 species that are distributed globally throughout tropical and subtropical regions. Cucurbits are distinguished from their closer relatives by the presence of tendrils, a specialized organ that allows them to adopt a climbing habit. This family exhibits a complex evolutionary history and contains many species of agronomical value such as pumpkins and squashes (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), muskmelon (*Cucumis melo*), cucumber (*Cucumis sativus*), wax gourd (*Benincasa hispida*), bottle gourd (*Lagenaria siceraria*), chayote (*Sechium edule*), bitter melon (*Momordica charantia*), and loofahs (*Luffa* sp.) (Renner and Schaefer, 2016). Previous studies uncovered two WGD events in this family: a cucurbit-common tetraploidization event (CCT) that predated (and possibly contributed to) the origin of the family (Wang et al., 2018), and a more recent allotetraploidization event shared by all species of *Cucurbita* (Sun et al., 2017; Montero-Pau et al., 2018). Barrera-Redondo et al. (2019) described in *Cucurbita* a link between this second WGD and faster rates of protein-coding and noncoding gene evolution, driving the emergence of molecular innovations. However, family-scale studies of WGDs in Cucurbitaceae were lacking. Furthermore, phylogenomic efforts to reconstruct a family-scale phylogeny for Cucurbitaceae have been hampered by conflicting signals between loci, suggesting that sampling more loci could help by discerning informative loci from noisy data (Bellot et al., 2020).

In this issue of *Molecular Plant*, Guo et al. (2020) used a phylogenomic approach including genomic and transcriptomic data from 136 cucurbit species (from which the data of 127 species were newly generated), covering 52 genera and all 15

tribes in the family. They identified four independent WGDs in the family, analyzed the changes in important morphological traits, and detected four peaks of diversification rates (Figure 1). The key contribution is the reconstruction of a well-supported phylogeny of Cucurbitaceae with a high taxonomic sampling using 1503 orthogroups of nuclear genes, recovering a phylogeny that is mostly congruent with previous studies (Renner and Schaefer, 2016), while resolving most of the phylogenetic positions at the tribe level that had low resolution in the phylogenomic study by Bellot et al. (2020). Guo et al. (2020) found two important differences in tree topology compared with previous studies: the relationships between Benincaseae, Coniandreae, and Cucurbiteae, and a difference in the position of Actinostemmatae as a basal group alongside Gomphogyneae. The phylogenetic position of Indofevilleae remains unresolved, as two of the topologies retrieved by Guo et al. (2020) placed it as a sister group of Thladiantheae, while the other four topologies placed Indofevilleae as the basal tribe of Cucurbitoideae, as previously reported by Renner and Schaefer (2016). The phylogenetic relationships reconstructed by Guo et al. (2020) agree with several morphological traits shared between the tribes in Cucurbitaceae, further supporting their results. However, as Bellot et al. (2020) suggest a more conservative approach by evaluating phylogenetic signals against noise across genomic datasets, future phylogenomic studies should be careful and assess noisiness before performing phylogenetic analyses.

After estimating the divergence times in the phylogeny, Guo et al. (2020) compared the species tree with 20 023 gene trees, finding evidence of four independent WGDs (Figure 1). The CCT was retrieved in this analysis at the origin of the Cucurbitaceae family, in accordance with Wang et al. (2018). The allotetraploidization event previously found in *Cucurbita* (Sun et al., 2017; Montero-Pau et al., 2018) was also detected, but unlike previous studies, the data of Guo et al. (2020) suggest that this WGD arose at the origin of the Cucurbiteae tribe rather than in *Cucurbita* (Figure 1). This WGD is supported by the larger haploid chromosome number in *Cucurbita* and *Sicana* ($n = 20$) in relationship with the ancestral karyotype in Cucurbitaceae of 15 chromosomes (Rice et al., 2015; Xie et al., 2019), as well as the two-to-one syntenic relationships between

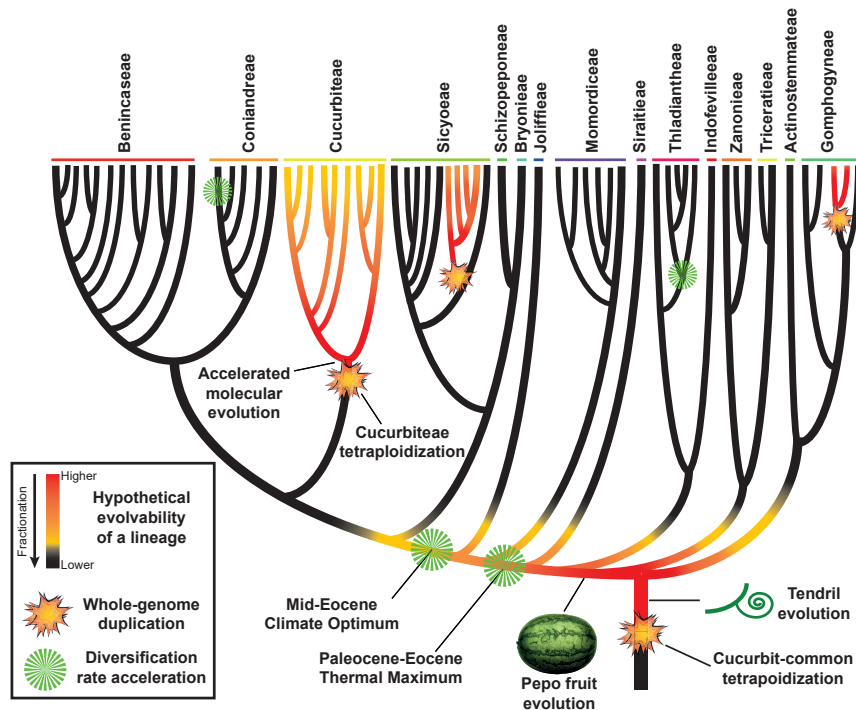


Figure 1. Simplified Schematic Diagram Showing the Evolutionary History of the Cucurbitaceae Family.

The Cucurbitaceae family experienced four independent whole-genome duplications according to a recent study by Guo et al. (2020). Among these duplications, the cucurbit-common tetraploidization event is hypothesized to have driven the evolutionary success of the family through an accelerated rate of molecular evolution that promoted, alongside specific paleoclimatic conditions, accelerations in the diversification rates and the emergence of key morphological traits such as the appearance of tendrils and the pepo fruit. Fractionation is the process of gene loss from a duplicated genomic region. The tree branches in the figure are not proportional to divergence times.

the *Cucurbita* chromosomes and the chromosomes of melon (Sun et al., 2017). Nonetheless, the genus *Cayaponia* of Cucurbitaceae shows a lower chromosome number than expected by this WGD ($n = 12$; Rice et al., 2015). Thus, future genome assemblies in other Cucurbitaceae genera will be needed to understand the extent of this WGD and its effects on karyotype evolution.

The other two WGDs were detected within five genera of the Sicyoeae tribe and among members of the *Hemsleya* genus (Figure 1). The evidence for these two WGDs is weaker, due to the lack of genome assemblies for these groups. Unlike *Cucurbita*, the haploid chromosome number of the five Sicyoeae genera ranges from 12 to 16, similar to the predicted ancestral karyotype of Cucurbitaceae (Rice et al., 2015; Xie et al., 2019). Meanwhile, some species within *Hemsleya* display a haploid chromosome number of 14, while others have 21 chromosomes (Rice et al., 2015); thus, this WGD probably took place within a few species rather than in the entire genus. Chromosome-level genome assemblies and further sampling of these groups will help us to understand these WGD events.

Guo et al. (2020) reconstructed the evolutionary transitions of several morphological traits, some of which are related to interesting ecological shifts associated with changes of pollinators, seed dispersal, and root storage. The burst of neofunctionalization processes previously observed in *Cucurbita* after its allotetraploidization event (Barrera-Redondo et al., 2019) seem to have also happened in the CCT that drove morphological novelty and diversification in Cucurbitaceae (Figure 1). The advent of the Cretaceous–Paleogene boundary and the warm periods in the Paleocene/Eocene possibly opened new ecological niches for these highly evolvable

cucurbits, promoting morphological transitions and increasing diversification rates.

Guo et al. (2020) were interested in the evolution of tendrils, as the emergence of climbing habits likely played an important role in the diversification and evolutionary success of Cucurbitaceae. The emergence of tendrils was apparently driven by the duplication and neofunctionalization of the *TEN* gene after the CCT (Figure 1). Two additional copies of the *TEN* gene were found in *Cucurbita* due to their second WGD, although the function of these additional copies is still elusive. However, neither *Hemsleya* nor *Echinocystis* show additional copies of *TEN*, as would be expected by their WGDs. This could reflect either a loss of these copies or the unavoidable incompleteness of transcriptomic datasets, as the additional *TEN* copies could have not been transcriptionally active during RNA extraction.

Other questions remain, such as how and why the fractionation processes reduced the cucurbit genomes back to sizes similar to those before their WGD events, or why the number of genes remains fairly constant in the family. Nonetheless, Cucurbitaceae stands out as an example of how WGDs underlie evolutionary novelty in plant species and, as more data continue to appear, WGDs might eventually be consolidated to be considered major drivers of plant evolution.

AUTHOR CONTRIBUTIONS

J.B.-R., R.L.S., and L.E.E. wrote the manuscript.

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Spotlight

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