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Hybridization History and Repetitive Element Content in the Genome of a Homoploid Hybrid, *Yucca gloriosa* (Asparagaceae)

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Hybridization in plants results in phenotypic and genotypic perturbations that can have dramatic effects on hybrid physiology, ecology, and overall fitness. Hybridization can also perturb epigenetic control of transposable elements, resulting in their proliferation. Understanding the mechanisms that maintain genomic integrity after hybridization is often confounded by changes in ploidy that occur in hybrid plant species. Homoploid hybrid species, which have no change in chromosome number relative to their parents, offer an opportunity to study the genomic consequences of hybridization in the absence of change in ploidy. *Yucca gloriosa* (Asparagaceae) is a young homoploid hybrid species, resulting from a cross between *Yucca aloifolia* and *Yucca filamentosa*. Previous analyses of ~11 kb of the chloroplast genome and nuclear-encoded microsatellites implicated a single *Y. aloifolia* genotype as the maternal parent of *Y. gloriosa*. Using whole genome resequencing, we assembled chloroplast genomes from 41 accessions of all three species to re-assess the hybrid origins of *Y. gloriosa*. We further used re-sequencing data to annotate transposon abundance in the three species and mRNA-seq to analyze transcription of transposons. The chloroplast phylogeny and haplotype analysis suggest multiple hybridization events contributing to the origin of *Y. gloriosa*, with both parental species acting as the maternal donor. Transposon abundance at the superfamily level was significantly different between the three species; the hybrid was frequently intermediate to the parental species in TE superfamily abundance or appeared more similar to one or the other parent. In only one case—*Copia* LTR transposons—did *Y. gloriosa* have a significantly higher abundance relative to either parent. Expression patterns across the three species showed little increased transcriptional activity of transposons, suggesting that either no transposon release occurred in *Y. gloriosa*

upon hybridization, or that any transposons that were activated via hybridization were rapidly silenced. The identification and quantification of transposon families paired with expression evidence paves the way for additional work seeking to link epigenetics with the important trait variation seen in this homoploid hybrid system.

Keywords: homoploid, hybrid, *Yucca*, chloroplast, transposable element, genomic shock

INTRODUCTION

Hybridization between related species has the potential to generate novel genotypic and phenotypic combinations, sometimes resulting in the origin of new species. Understanding the factors that promote the process of hybridization, as well as the maintenance of newly created hybrids, has been of considerable interest to both the fields of ecology and evolution (Gross and Rieseberg, 2005). As the generation of biodiversity is of primary importance to evolutionary biology, many studies have sought to determine whether or not newly created hybrids are reproductively isolated from parental species and are capable of persisting in a hybrid state for many generations. The tools aimed at studying plant hybridization include observational studies of plants and their pollinators in the wild (Leebens-Mack and Milligan, 1998; Hersch and Roy, 2007), reciprocal transplant studies across multiple environments (Wang et al., 1997), manual pollinations between related species (Sun et al., 2018), cytogenetics (Thórsson et al., 2001), and population genomics (Bredeson et al., 2016). Hybridization can result in allopolyploid individuals, in which hybridization occurs at the same time as chromosome doubling, as well as homoploids, in which there is no change in chromosome number (for a review, see Rieseberg, 1997; Soltis and Soltis, 2009). Transposable element content and abundance has been hypothesized to contribute to genome dominance in allopolyploid species (Edger et al., 2017; Bird et al., 2018), but change in ploidy makes it difficult to assess its importance relative to hybridization in the genesis of a new species. Homoploid hybrid species provide an opportunity to focus on the effects of hybridization while controlling for ploidy level (Ungerer et al., 2009; Staton et al., 2012).

Investigation of hybridization almost always begins with a detailed understanding of the genetics and life history of the putative parental and hybrid species. In the case of wild sunflowers, numerous studies have focused on how *Helianthus annuus* and *H. petiolaris* have hybridized multiple independent times to form three homoploid hybrid species: *H. anomalus*, *H. deserticola*, and *H. paradoxus* (Rieseberg, 1991; Rieseberg et al., 2003). These hybrid species are morphologically distinct from their parents and each other (Rieseberg et al., 2003), display varying levels of salt tolerance (Welch and Rieseberg, 2002; Karrenberg et al., 2006), show gene expression differences (Lai et al., 2006), and exhibit population genetic patterns consistent with selective sweeps (Sapir et al., 2007). The repeated formation of homoploid hybrids in *Helianthus* has increased our understanding of hybrid speciation from both ecological and genomic perspectives, yet it is only one example of homoploid hybridization in flowering plants. Another well-studied example of homoploid hybridization is in *Iris nelsonii*,

a hybrid suspected to have genetic contributions from more than two species based on patterns of both nuclear and plastid genetic variation (Arnold, 1993). The fitness of the hybrid species relative to the parental species varies depending on the moisture of the environments, implying that genotype-by-environment interactions differentially affect parental and hybrid genotypes, a phenomenon that can lead to hybrid speciation (Johnston et al., 2001).

While hybridization's effect on the generation of biodiversity and the movement of adaptive traits between species has been well established, the effect on the genome is only recently being fully understood. McClintock (1984), described hybridization as a "challenge" or "shock" for the genome; the merger of two separate genomes in a single nucleus results in a completely novel genomic environment. Post hybridization, alleles once restricted to separate species now interact in a new cellular setting, allowing for the formation of novel phenotypes, epistatic interactions, and potentially significant and rapid evolutionary change. Possible outcomes of hybridization and subsequent genome shock include: alteration of gene expression (Hegarty et al., 2009; Xu et al., 2009); chromosomal rearrangements (Rieseberg et al., 1995; Lai et al., 2005; Danilova et al., 2017); genome dominance, in which one progenitor genome expresses and/or retains more genes (Rapp et al., 2009; Bardil et al., 2011; Schnable et al., 2011; Yoo et al., 2013; Edger et al., 2017; Bird et al., 2018); epigenetic perturbation (Salmon et al., 2005), which in turn can lead to a release of silencing of repetitive elements and allows for subsequent repeat proliferation (Ungerer et al., 2006; Parisod et al., 2009).

Repetitive elements in particular have been implicated in the divergence of hybrid species from their progenitors. For example, RNA-seq suggests that established homoploid hybrid sunflowers, as opposed to newly synthesized hybrids, have elevated transposon expression levels (Renaut et al., 2014). In two of these hybrid sunflower species fluorescent *in situ* hybridization studies identified expansions of *Gypsy* retrotransposons relative to the progenitor species (Staton and Ungerer, 2009). *Gypsy* and *Copia* elements are typically the most abundant superfamilies in plant genomes, and are both Class I retrotransposons that replicate via a "copy and paste" mechanism (Wessler et al., 1995), in contrast to the variety of Class II DNA transposons that replicate via a "cut and paste" mechanism (Feschotte and Pritham, 2007). Transposons can affect traits by disrupting genes, duplicating or re-organizing genes (Xiao et al., 2008), or they can land upstream and create new patterns of gene expression (Studer et al., 2011). The accumulation of transposons contributes to a large proportion of genome size variation seen in plants (Tenailon et al., 2011), and ectopic recombination between transposable elements can result in

genomic deletions and are a major force in genome evolution (Devos et al., 2002).

While homoploid hybrid systems are relatively rare, recent efforts to sequence the genomes of *Yucca* (Asparagaceae) species allows us to investigate the effects of hybridization on a homoploid genome. *Yucca aloifolia* L. and *Yucca filamentosa* L. are emergent models in understanding the evolution of CAM photosynthesis, as the species use CAM and C₃, respectively (Heyduk et al., 2016). The two species also hybridize to form *Y. gloriosa* L. (Rentsch and Leebens-Mack, 2012), which is photosynthetically intermediate and a relatively recently derived homoploid hybrid species (Trelease, 1902). All three species have genome sizes of ~2.8 Gb (Heyduk unpublished) and are sympatric in the Southeastern United States, with *Y. filamentosa* found across a broader range of the eastern seaboard, including into New England and the Midwest; *Y. aloifolia* is restricted largely to the Southeastern United States and reaches only as far north as North Carolina. *Yucca gloriosa* is even more restricted than either parent in its range, found only in the coastal dune systems of the Atlantic seaboard and, based on herbarium records, along the coast of the Gulf of Mexico. It is thought that *Y. aloifolia* was introduced into the Southeastern United States from Mexico or the Caribbean by Spanish colonists (Trelease, 1902; Groman and Pellmyr, 2000). Perhaps as a result of the human-involved introduction, *Y. aloifolia* has escaped the dependence on the obligate *Yucca-yucca* moth pollination mutualism and can be pollinated by the yucca moth *Tegeticula yuccasella* (Leebens-Mack and Pellmyr, 2004) or introduced generalist honeybees (*Apis mellifera*) (Rentsch and Leebens-Mack, 2014). *Yucca filamentosa* still retains its obligate pollination mutualism with the yucca moths (*Tegeticula yuccasella* and *T. cassandra*) (Pellmyr, 1999), and overlaps in flowering time with *Y. aloifolia* briefly and only in some years, suggesting that hybridization between the two species may be rare.

Previous work suggested no variation in chloroplast or microsatellite repeats in a small sampling of *Y. aloifolia* genotypes, and further indicated that *Y. aloifolia* is the maternal parent in any hybridization events that led to *Y. gloriosa* (Rentsch and Leebens-Mack, 2012). Through a whole genome sequencing project that aims to assemble the genomes of *Y. aloifolia* and *Y. filamentosa*, resequencing was performed on individuals of all three *Yucca* species. Using the resequencing data, we sought to re-test hypotheses on the number and direction of hybridization events in *Y. gloriosa*. Specifically, we assembled maternally inherited chloroplast genomes, which can inform not only the evolutionary history, but also the direction of hybridization. We assessed whether all hybrid *Y. gloriosa* individuals were nested within *Y. aloifolia* on a phylogenetic tree and haplotype network, consistent with the hypothesis of a single hybridization event with *Y. aloifolia* as the maternal parent. We further examined the repeat landscape of all three species to determine if repeat content in the hybrid is intermediate between the two parents, or if transgressive repeat abundance exists, suggesting a degree of post-hybridization genomic shock. Finally, using existing RNA-sequencing datasets in the three species of *Yucca*, we examined the activity of repeats using mRNA reads as a proxy. Through

the use of high throughput genomic data, we find that *Y. gloriosa* is the result of repeated and bi-directional hybridization events that evidently led to minimal repeat proliferation. Our findings further suggest that there is little evidence of repetitive element release in *Y. gloriosa* as a result of hybridization.

MATERIALS AND METHODS

DNA Sampling, Library Preparation, and Sequencing

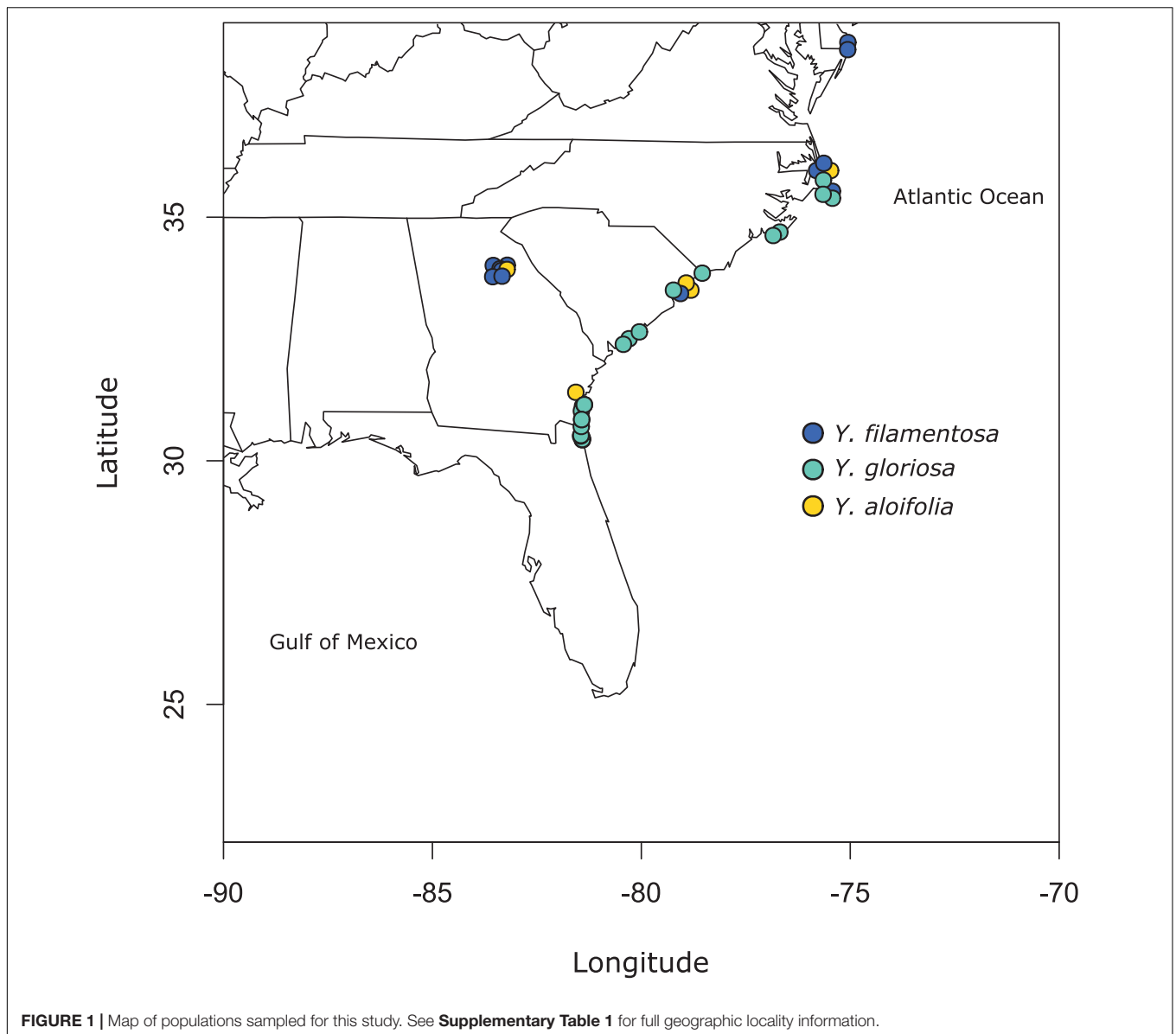
Clones of 41 individuals (5 from *Y. aloifolia*, 24 from *Y. gloriosa*, and 12 from *Y. filamentosa*) were collected throughout the Southeastern United States from 2013 to 2015 and planted in the University of Georgia greenhouse (Figure 1 and Supplementary Table 1). In 2018, approximately 100 mg of fresh tissue was harvested from fully expanded leaves and kept on ice until DNA extraction, using a CTAB protocol with sorbitol addition that removes secondary compounds before DNA purification (Doyle, 1987; Štorchová et al., 2000). DNA was visualized on a 1.5% agarose gel to measure integrity and quantified via Qubit. Samples were shipped to the HudsonAlpha Institute for Biotechnology, where Illumina 350 basepair PCRfree fragment libraries were constructed using standard protocols. Each library was uniquely barcoded and sequenced on a NovaSeq 6000 with paired end 150bp reads. Data is available on the NCBI Sequence Read Archive (for a full list of SRA accessions, see Supplementary Table 1).

Chloroplast Genome Assembly and Analysis

Raw reads were first quality trimmed using Trimmomatic v 0.36 (Bolger et al., 2014). Due to the sheer size of the sequence data per individual—roughly 400–800 million reads—a subset of four million paired-end reads was randomly sub-sampled from each library's trimmed dataset in order to speed up computational analyses. The sub-sampled data were used as input into the program Fast-Plast¹, which assembles plastid genomes by first mapping reads to a reference plastid genome (here we used a previously assembled *Y. filamentosa* chloroplast genome; McKain et al., 2016).

Chloroplast genomes of *Agave americana* (NCBI accession: KX519714.1, Abraham et al., 2016) and *Nolina atopocarpa* (NCBI accession: NC_032708.1) were used as outgroups for phylogenetic analyses. All *Yucca* chloroplast assemblies as well as *Agave* and *Nolina* were aligned using MAFFT (Katoh and Standley, 2013). The alignment was manually inspected for misaligned regions, and as a result three *Yucca* genotypes (*Y. aloifolia* YA7, and *Y. gloriosa* YG13 and YG61) containing considerable misalignments indicative of a sub-optimal genome assembly were not included in further analyses. The second inverted repeat (IR) region was removed before tree estimation: an aligned *Y. aloifolia* chloroplast genome sequence (YA23) was annotated for the IR by conducting a

¹<https://github.com/mrmckain/Fast-Plast>



BLASTn (Altschul et al., 1990) against itself. The position of an inverted self-hit in YA23 was used to remove the second IR from the multi-species alignment. The optimal model of molecular evolution (GTR + Gamma) was determined using JModelTest v2 and BIC penalized-likelihood (Darriba et al., 2012) on the CIPRES gateway (Miller et al., 2010). The multiple sequence alignment was then used to estimate a chloroplast phylogeny using RAxML v8.2.11, with 500 bootstrap replicates (Stamatakis, 2006). The entire chloroplast alignment (with both IR) of the *Yucca* species without outgroups was also used to construct a median joining chloroplast haplotype network using PopArt (epsilon = 0) (Leigh and Bryant, 2015). Chloroplast genome assemblies were annotated in Geneious Prime 2019.2.3, using the built-in annotation tool with the previously published *Y. filamentosa* annotation as a reference (NCBI accession: KX931467, McKain et al., 2016). Chloroplast genome assemblies

are available on NCBI's GenBank (**Supplementary Table 1**), and the plastid alignment and newick files can be found on github².

Repetitive Content Classification and Analysis

In a similar fashion to the chloroplast sequence processing, one million trimmed paired-end reads were randomly sub-sampled for an analysis of transposon content. In order to ensure that only nuclear repetitive sequences were being analyzed, reads were first mapped to *Yucca* chloroplast and mitochondrial genome sequences (reference files are available at JGI Genome Portal³) using Bowtie v2 with default settings (Langmead and Salzberg, 2012) to be flagged for removal. The nuclear data were retained

²https://github.com/kheyduk/Yucca_plastome

³<https://genome.jgi.doe.gov>

and further processed in preparation for downstream steps, including: converting bam mapping files to fastq files using SAMTools v1.9 (Li et al., 2009) and BEDTools v2.26 (Quinlan and Hall, 2010), interleaving fastq files so that pairs are found sequentially in a single file (script⁴, from Boisvert et al., 2010), and converting fastq files to fasta files with the FASTX-Toolkit v 0.14⁵.

Transposome (Staton and Burke, 2015) was used to cluster and identify repetitive DNA sequences in all 41 *Yucca* genotypes using a *Yucca*-specific reference. Briefly, RepeatModeler (Smit and Hubley, 2008) was used to predict repeat families *de novo* on the assembled *Yucca* genomes; RepeatModeler uses both RECON (Bao and Eddy, 2002) and RepeatScout (Price et al., 2005) to identify repeat family consensus sequences. To remove false positives (e.g., repetitive domains within genes), the predicted RepeatModeler consensus sequences were searched for functional PFAM and Panther domains. If no domains—or only known transposable element domains—were found in a given putative repeat family, it was retained as a true repeat; if only false positive domains were identified, the family was removed from further analysis. Putative repeat families that had a combination of transposable element and false positive domains, or had otherwise unknown domain classes, underwent manual curation.

For annotating *Y. aloifolia* and *Y. filamentosa* repeats via Transposome, we used the species-specific RepeatModeler families (repetitive element reference files are available at JGI Genome Portal; see footnote). For *Y. gloriosa* hybrid individuals, we concatenated the two parental repeat databases. Finally, we used the following parameters in our usage of Transposome: percent identity = 90%, a required fraction of overlap between pairwise matches of 0.55, a minimum cluster size of 100, a merge threshold of 1,000, and a BLAST e-value of 1. Cross-species comparisons of transposons included the average amount of total repetitive DNA as well as the relative amounts (genomic proportion) of transposon superfamilies. In R v. 3.6.1 and v. 4.0.2 (R Core Team, 2019), we used ANOVA to determine whether there were significant differences between species in the relative amount of repetitive DNA in each of the 10 transposon superfamilies and abundance of individual family lineages within a superfamily. *Post hoc* tests were conducted with the emmeans package in R⁶. Additionally, a data matrix containing each individual's relative amount of repetitive DNA for each of the 10 superfamilies served as the input for a principal components analysis, using the prcomp() function in R. Throughout, we use the transposable element classification system described in Wicker et al. (2007).

Repetitive Element Activity via mRNAseq

Many repetitive elements contain sequences that are involved in their replication and therefore are transcribed into mRNA; transcripts produced from these repeats can be detected by mRNA sequencing (Hollister et al., 2011; Dion-Côté et al., 2014). While read counts from mRNA sequencing are a proxy for transcription of a repeat, no assumptions can be made as to

the successful integration of a repeat copy into the genome post transcription; a variety of genomic mechanisms exist to silence and degrade repetitive element-derived transcripts (Lisch, 2009; Fultz et al., 2015). Nevertheless, as a first approximation of repeat activity, we used previously published mRNA-seq data on the three species of *Yucca* analyzed here (Heyduk et al., 2019). Briefly, RNA from leaf tissue was collected from all three species of *Yucca* growing in growth chambers set to 30°C/18°C day/night temperatures, with ~400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of light at leaf level, and 40% humidity in a 12 h day/night light regime. While the previous study further assessed gene expression under drought, here only libraries from well-watered plants taken during the daytime were analyzed. The original study used 2–3 genotypes per species, each of which had 2–3 replicates that were taken from different time points during the day. Because replication within a genotype is confounded with time, we limited our analyses to considering only species-specific differences rather than examining genotypic differences within species. Final species-level replication varied from 6 in *Y. aloifolia* to 9 in *Y. gloriosa* and *Y. filamentosa*.

RNA reads were mapped to the same repeat databases used in Transposome; *Y. aloifolia* and *Y. filamentosa* reads were mapped to each species' specific repeat reference, while *Y. gloriosa* reads were mapped to a merged parental reference. RNA reads were mapped via Kallisto v 0.43 using default parameters (Bray et al., 2016). For *Y. gloriosa*, counts were summed in cases where both parental species had a consensus sequence for a given repeat family. Libraries were first normalized by the Trimmed Mean of M-values (TMM) (Robinson and Oshlack, 2010) as implemented in EdgeR (Robinson et al., 2010), then scaled by overall abundance of that repeat family as estimated by Transposome. To scale, a matrix consisting of all repeat abundances across all genotypes from the three *Yucca* species was scaled by the maximum abundance of all families identified by Transposome. These scaled abundance values were then used as a multiplier of the TMM normalized read counts. By normalizing by genomic abundance, expression of repeats could then be compared across genotypes and species that have varying genomic fraction of the repeat families. Once normalized and scaled, we tested for significant expression within species using a glm intercept model in the glm.nb() function in the R package MASS (Venables and Ripley, 2013), which employs a negative binomial model appropriate for count data that exhibits a degree of over dispersion. Differentially expressed repeats between species were also tested with a negative binomial model, and *post hoc* tests were done using the emmeans() function from the R package emmeans.

RESULTS

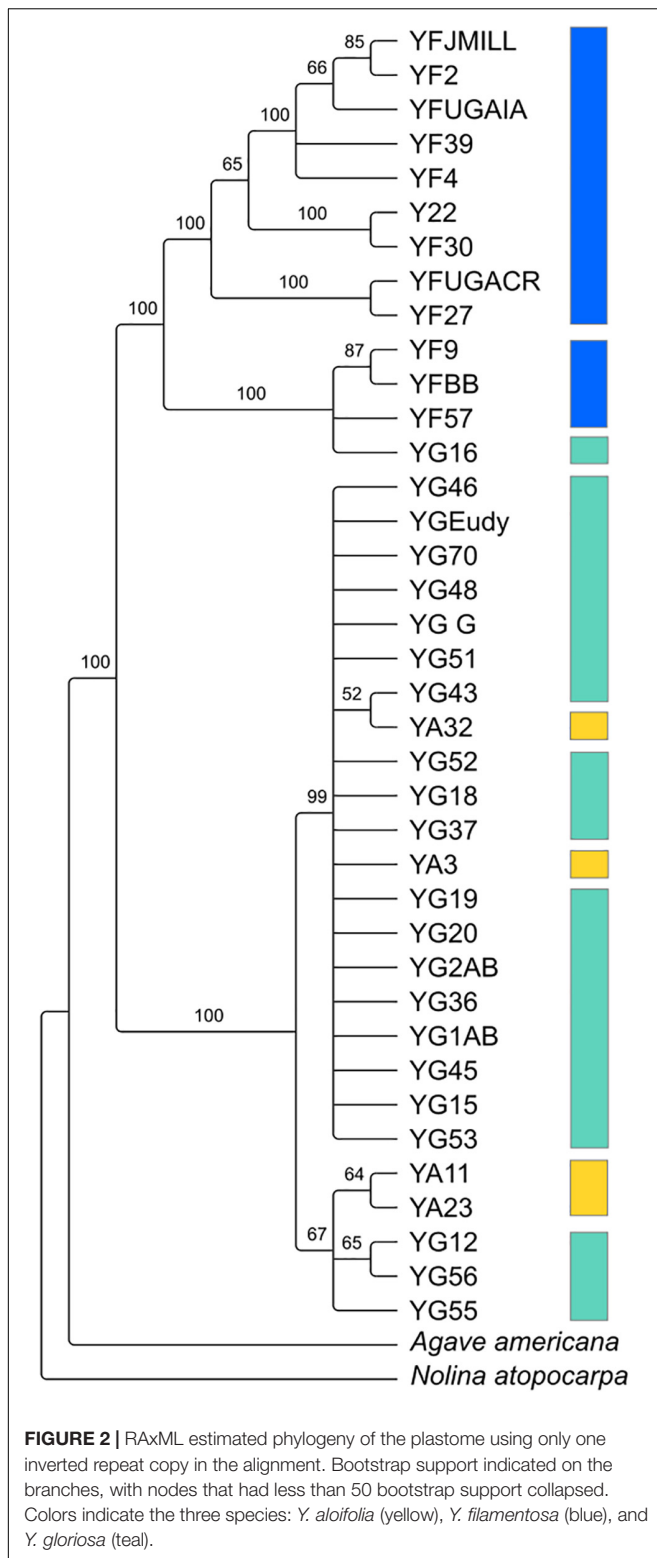
Plastid Phylogenetic and Haplotype Analyses

Despite the relatedness between the three *Yucca* species studied here, there was enough divergence between the species' chloroplast genomes to identify highly supported clades of chloroplast haplotypes (Figure 2). *Y. gloriosa* genotypes were

⁴<https://github.com/sebhtml/interleave-fastq.py>

⁵http://hannonlab.cshl.edu/fastx_toolkit/

⁶<https://github.com/rvlenth/emmeans>



found nested within three separate clades (Figure 2). A single *Y. gloriosa* genotype, YG16, was within a clade that otherwise contained all of the *Y. filamentosa* individuals that were analyzed. Three *Y. gloriosa* genotypes (YG12, YG55, and YG56) were

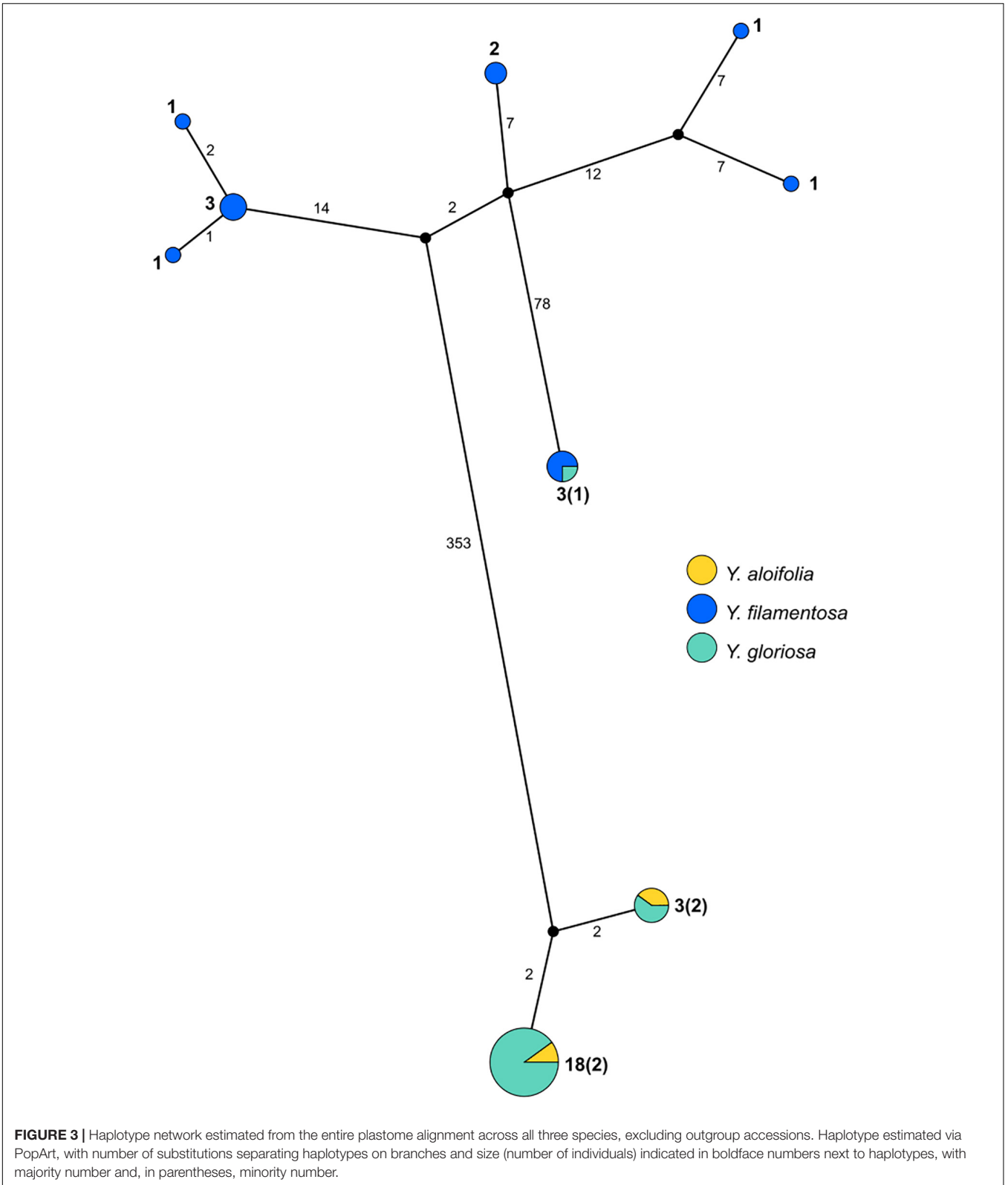
placed in a clade with two *Y. aloifolia* genotypes (YA23 and YA11). The remaining 18 *Y. gloriosa* genotypes were grouped with the remaining two *Y. aloifolia* individuals (YA3 and YA32).

PopArt haplotype analysis (Leigh and Bryant, 2015) identified the same patterns found in the maximum likelihood-based phylogeny. Over 350 substitutions differentiated the two major groupings of genotypes (*Y. aloifolia* and *Y. filamentosa*-like chloroplast genomes; Figure 3). *Yucca filamentosa* had considerably more chloroplast haplotypes compared to *Y. aloifolia* (7 vs. 2, respectively; Figure 3). In contrast to previous analysis of nuclear simple repeats (Rentsch and Leebens-Mack, 2012), genetic diversity was seen not only in the *Y. aloifolia* chloroplast genomes but also for *Y. gloriosa*, which had four substitutions separating the different *Y. aloifolia*-like haplotypes, and over 400 substitutions separating the single *Y. filamentosa*-like haplotype from other individuals of *Y. gloriosa*.

Repetitive Fraction of *Yucca* Genomes

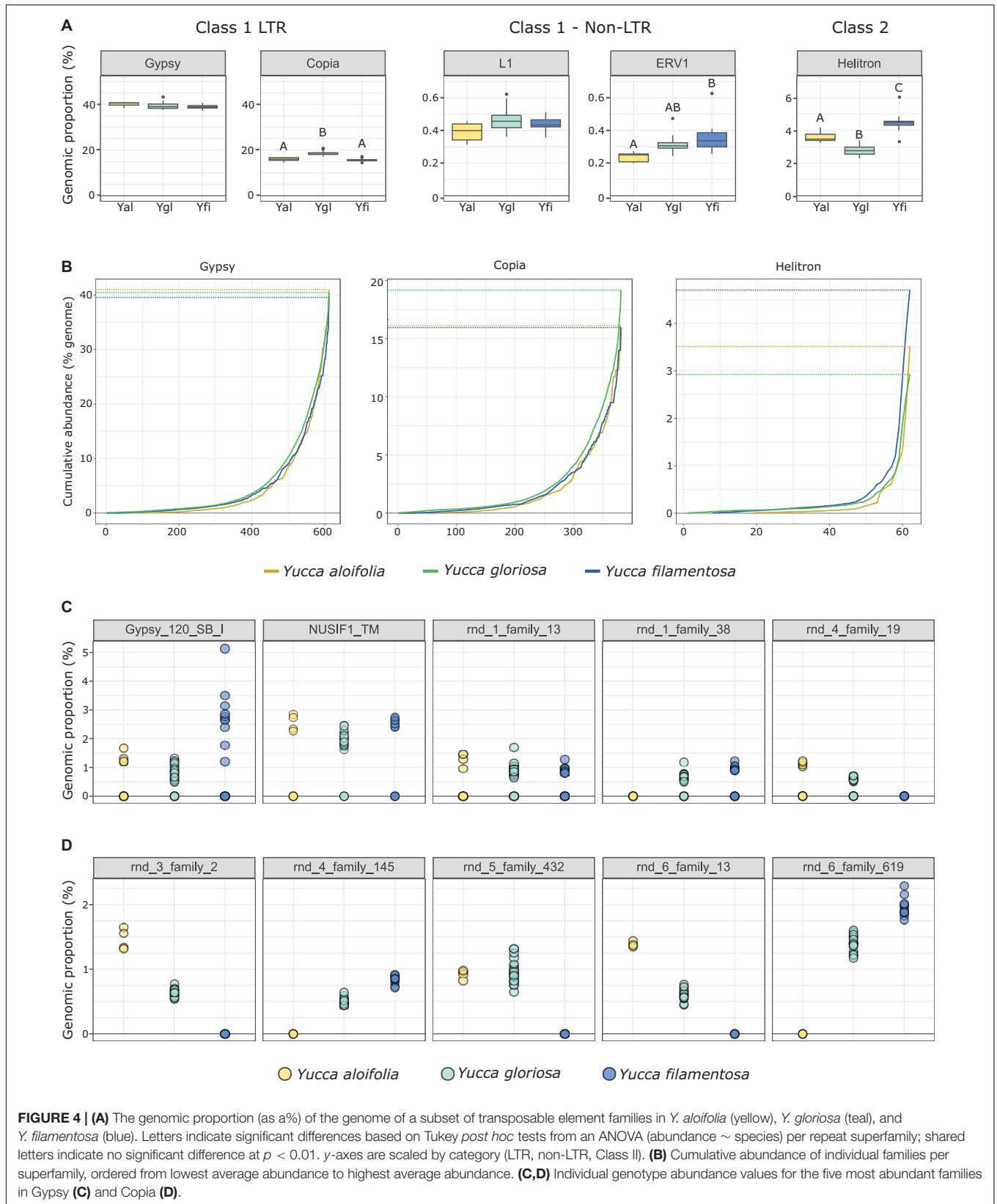
The fraction of the genome containing repetitive DNA significantly differed between the three species [$p < 0.001$, $F_{(2, 38)} = 17.853$]. While *Y. aloifolia* (mean repetitive genome fraction = 0.658; $SD = 0.0138$) and *Y. gloriosa* (mean = 0.662; $SD = 0.0215$) had statistically indistinguishable amount of repetitive DNA, *Y. filamentosa* was significantly lower than both species (mean = 0.621; $SD = 0.0167$; $p < 0.01$ for both *post hoc* comparisons). Moreover, the fraction of the genome composed of the various repeat families varied across the three species. The most abundant type of repeat in all three genomes were members of the *Gypsy* superfamily (Figure 4A), comprising ~39% of the total genome, although species did not significantly differ in overall *Gypsy* abundance. The second most abundant superfamily in the *Yucca* genomes, at about ~16.5%, was *Copia* (Figure 4A). *Yucca gloriosa* had significantly more *Copia* elements than either parent (*post hoc* comparison of *Y. gloriosa* to either parent $p < 0.001$). The third most abundant repeat superfamily was DNA *Helitrons*, at ~3.5%, which had significantly different abundances between all three species (*post hoc* comparison $p < 0.01$). In general, the variation in repeat superfamily abundance between the three species was large enough to distinguish each species (Supplementary Figure 1), though intraspecific variation in repeat abundance was apparent as well. Certain transposable element superfamilies not typically seen in plant genomes—including non-LTR *Zisupton*, *Novosib*, and *Line-2* (*L2*) elements—were found at non-zero abundance levels in the *Yucca* species (Supplementary Table 2), but are not considered further here, as they may be the result of contamination in DNA isolation (e.g., from fungi).

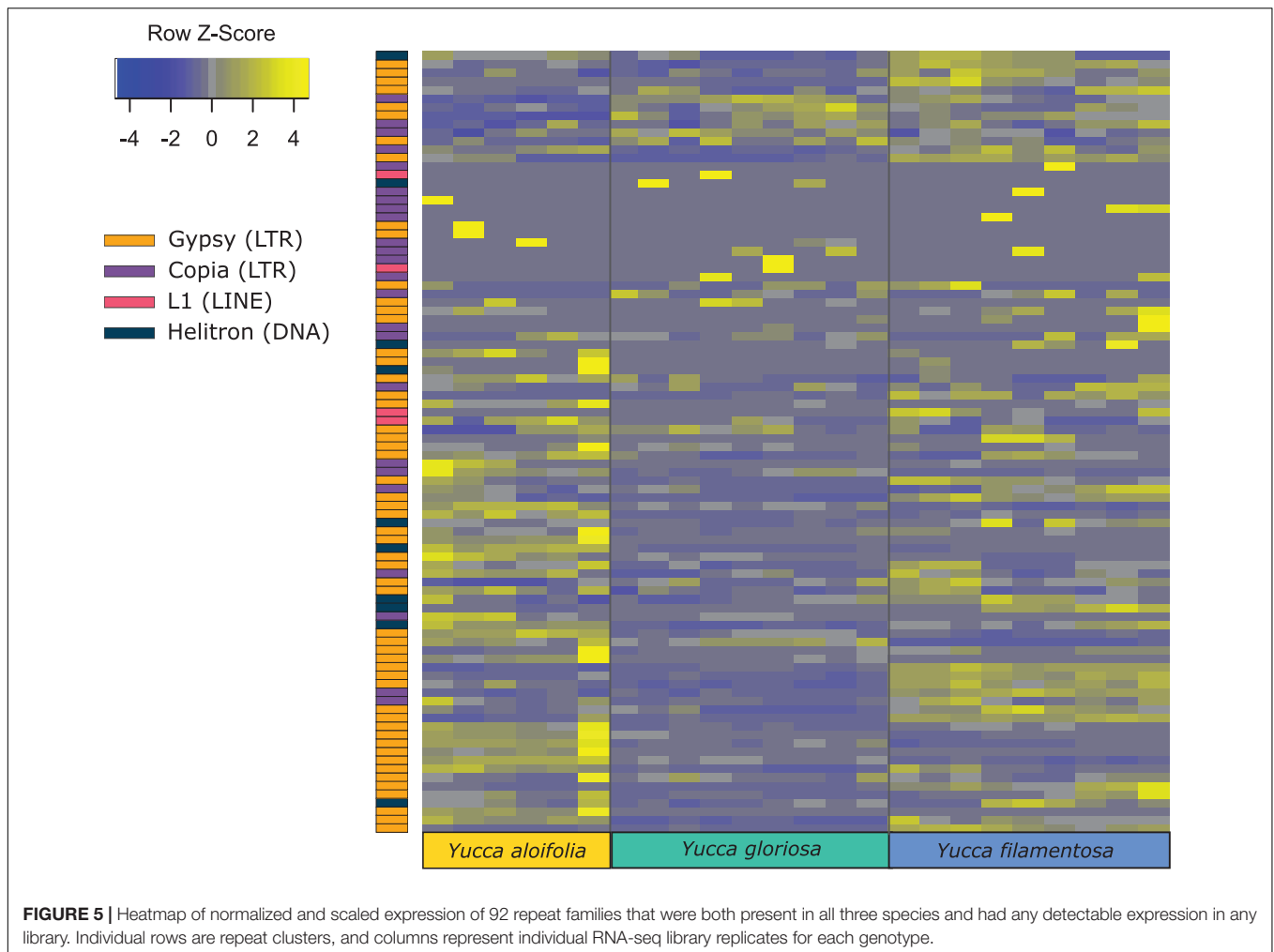
At the family level (repeat lineages within a superfamily), while variation across the three species existed (Supplementary Table 3), it was rare that a single family contributed to the differences seen at the superfamily level (Figure 4A). In particular, high abundance families in *Copia* showed a pattern of parental-specific ancestry, in that they were found in only one of the two parental species. Furthermore, these high abundance, parental-specific repeat families also had high



abundance inherited in the hybrid, resulting in an overall higher abundance of *Copia* elements in *Y. gloriosa* relative to either parent (Figures 4B,C). In contrast, high abundance *Gypsy*

elements were not parental-specific, and the hybrid therefore had equivalent abundance values as the parental abundance (Figures 4B,C). These high abundance, parental-specific families





in the *Copia* superfamily drive the overall species difference at the superfamily level.

Repeat mRNA Expression

Transposome abundance analysis of *Y. aloifolia* and *Y. filamentosa* identified 504 and 726 repeat families in at least one genotype of either species, respectively; only 231 repeat families were present in both parental species. Of the 231 families present in both species, only 118 and 119 repeat families had significantly non-zero expression in *Y. aloifolia* and *Y. filamentosa*, respectively (Benjamini-Hochberg adjusted $p < 0.01$) (**Supplementary Table 4**), and only 92 families were both present in all three species and had detectable expression in any one library (**Figure 5**). Only 27 families were significantly expressed in both parental species (**Table 1**). Repeat families with significant expression were typically from *Gypsy* (64 and 61% of total families expressed in *Y. aloifolia* and *Y. filamentosa*, respectively) and *Copia* (25, 27%) superfamilies. *Yucca gloriosa* had largely overlapping expression with its parental species; the hybrid shared significant expression of 74 families with *Y. aloifolia* and 70 families with *Y. filamentosa*. *Yucca gloriosa* had only two families that were not also significantly expressed

in either parent: one a member of the *Gypsy* superfamily, the other belonging to the *Copia* superfamily, and both had genomic abundance at less than 1%. Overall, normalized and scaled expression values were positively correlated with the genomic abundance of a family across the three species [$r = 0.23$, $t_{(5, 214)} = 4.9$, $p < 0.001$ for full data, $r = 0.41$, $t_{(5, 204)} = 31.83$, $p < 0.001$ for families with TPM < 2,000].

In comparing the repeat families that are significantly expressed in *any* of the three species, *Y. gloriosa* showed little transgressive expression patterns; in most of the 178 repeat families that had significant *post hoc* comparisons, *Y. gloriosa* was not statistically different than one of its parental species. There were only three repeat families where expression differed significantly in all three species (*post hoc* $p < 0.01$) (**Supplementary Table 5**), and in 5 families, *Y. gloriosa* exhibited an expression level that was significantly different than the pattern shared in the two parental species (*post hoc* $p < 0.01$) (**Figure 6**). In all five cases, *Y. gloriosa* expression was significantly lower than the parental species' expression, though notably not zero. In general, however, the expression levels of repetitive elements in *Y. gloriosa* were shared with one or both parental species. Nine transposons families showed shared expression

TABLE 1 | Mean expression and abundance of repeat families in *Y. aloifolia* and *Y. filamentosa* significantly expressed above zero ($p < 0.01$).

Family	Superfamily	<i>Yucca aloifolia</i>		<i>Yucca filamentosa</i>		<i>Yucca gloriosa</i>	
		Mean Exp. ^a	Mean Abun. ^b	Mean Exp.	Mean Abun.	Mean Exp.	Mean Abun.
Copia_18_BD_I ^{§Φ}	LTR/Copia	15.10	0.01%	16.19	0.01%	5.37	0.004%
Copia12_ZM_I	LTR/Copia	4.49	0.15%	2.91	0.10%	1.86	0.08%
Gypsy_120_SB_I ^Φ	LTR/Gypsy	12.67	1.21%	24.94	2.62%	8.58	0.91%
Gypsy_3_OS_I ^Φ	LTR/Gypsy	16.52	0.12%	97.12	0.29%	9.82	0.09%
Gypsy_4_BD_LTR [§]	LTR/Gypsy	4.91	0.10%	4.16	0.10%	1.62	0.07%
Gypsy_5B_OS_LTR ^{§Φ}	LTR/Gypsy	66.37	0.53%	38.85	0.27%	14.00	0.19%
Gypsy_8_OS_I ^Φ	LTR/Gypsy	70.39	0.12%	372.53	0.34%	45.28	0.13%
Helitron_N117_OS ^Φ	Helitron	1796.11	0.76%	1953.53	1.19%	989.95	0.67%
Helitron_N29B_OS	Helitron	3.77	0.10%	6.11	0.11%	3.13	0.09%
Helitron_N84_OS ^{§Φ}	Helitron	16.65	1.20%	12.14	0.71%	4.67	0.46%
Helitron7_OS	Helitron	92.69	0.86%	104.54	1.03%	52.54	0.60%
NUSIF1_TM ^{§Φ}	LTR/Gypsy	507.41	2.80%	405.08	2.64%	128.68	1.87%
rnd_1_family_13 ^{§Φ}	LTR/Gypsy	146.24	1.22%	147.59	0.96%	65.60	1.03%
rnd_1_family_14 [§]	LTR/Gypsy	58.75	0.48%	17.27	0.17%	22.38	0.27%
rnd_1_family_15 ^{§Φ}	LTR/Gypsy	98.84	0.49%	21.90	0.35%	41.25	0.43%
rnd_1_family_20 ^{§Φ}	LTR/Gypsy	65.94	0.43%	20.69	0.15%	9.31	0.14%
rnd_1_family_23 ^Φ	LTR/Gypsy	15.88	0.11%	45.68	0.35%	12.38	0.17%
rnd_1_family_30 ^Φ	LTR/Gypsy	8.83	0.20%	14.89	0.28%	5.18	0.24%
rnd_1_family_32	LTR/Gypsy	7.75	0.09%	5.83	0.24%	4.97	0.17%
rnd_1_family_37	LTR/Gypsy	9.69	0.13%	8.24	0.10%	4.28	0.12%
rnd_1_family_47 [§]	LTR/Gypsy	22.26	0.50%	3.63	0.33%	5.91	0.51%
rnd_1_family_505 ^Φ	LTR/Copia	2.66	0.13%	20.96	0.36%	3.93	0.18%
rnd_1_family_56	LTR/Gypsy	66.87	0.21%	54.49	0.20%	58.95	0.25%
rnd_1_family_71 ^Φ	LTR/Gypsy	13.84	0.19%	3.05	0.35%	12.97	0.27%
rnd_1_family_76 [§]	LTR/Gypsy	56.40	0.28%	10.09	0.20%	13.02	0.23%
rnd_1_family_9 [§]	LTR/Gypsy	275.20	0.54%	15.66	0.11%	20.41	0.16%
SZ_22_int ^Φ	LTR/Gypsy	2.43	0.14%	9.69	0.25%	1.84	0.16%

^aMean expression is TMM normalized and scaled by abundance, then averaged across libraries.

^bMean abundance is the genomic fraction predicted by Transposome per genotype and averaged across genotypes within each species.

Indicates repeat family is significantly differentially expressed between the parental species (*), between *Y. gloriosa* and *Y. aloifolia* (§), or between *Y. gloriosa* and *Y. filamentosa* (Φ), all at $p < 0.01$.

See **Supplementary Table 5** for full test results and ANOVA statistics.

in *Y. gloriosa* and *Y. filamentosa* that differed significantly from *Y. aloifolia*, and seven transposons had shared expression between *Y. gloriosa* and *Y. aloifolia* that differed significantly from *Y. filamentosa*. The majority of transposons had shared expression between the two parents, but significantly different expression between *Y. gloriosa* and either *Y. aloifolia* ($n = 76$) or *Y. filamentosa* ($n = 77$). There was a single transposon family where the parental species had significantly different expression from each other and *Y. gloriosa*'s expression was not significantly different than either parent.

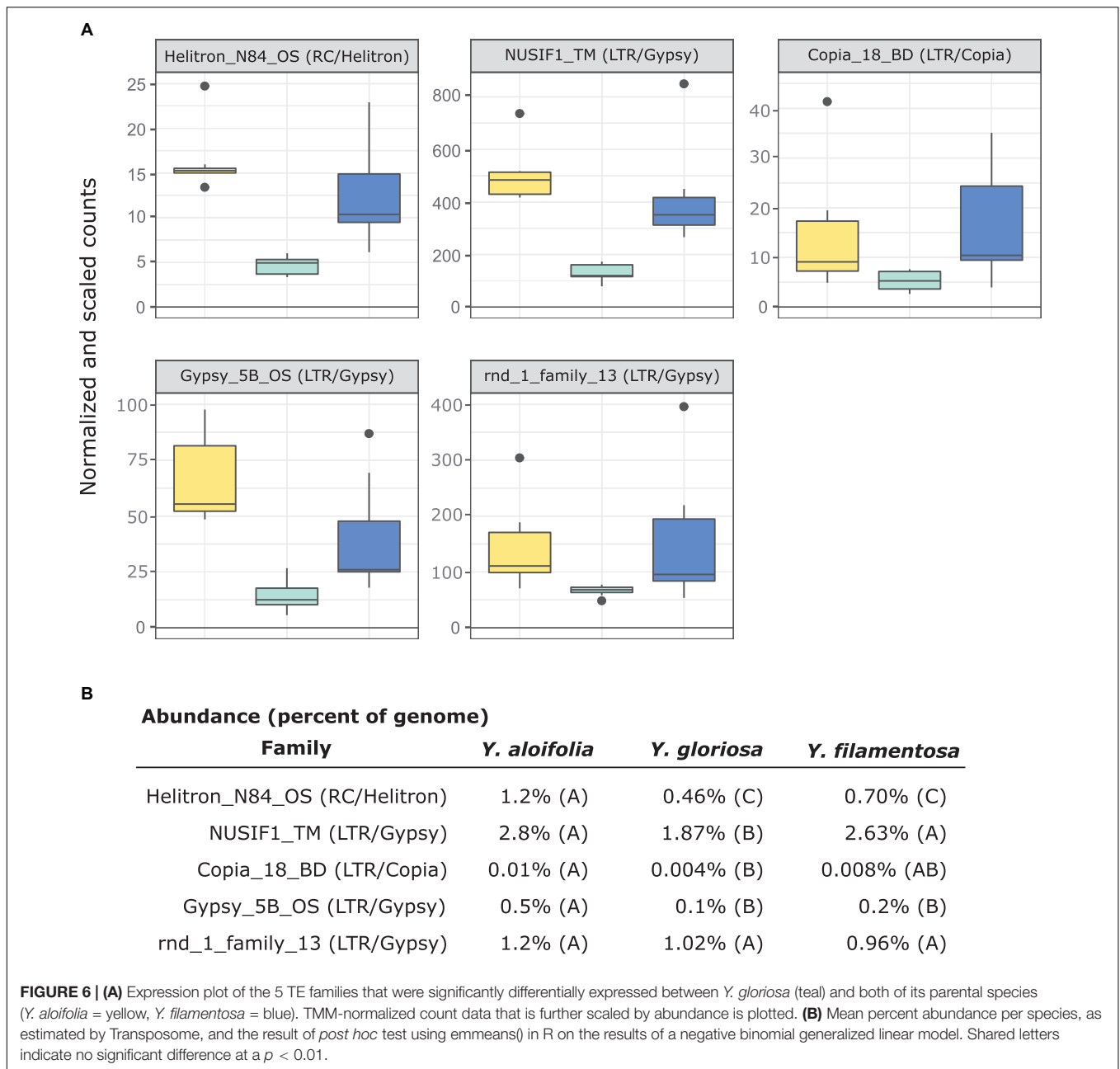
DISCUSSION

By increasing both the number of *Yucca* genotypes and assessing the whole chloroplast genome we have greatly improved resolution of the history of homoploid hybridization in *Yucca* relative to previous analyses of simple sequence repeats and short fragments of the chloroplast (Rentsch and Leebens-Mack, 2012). Whereas the previous work inferred a single, shared

plastid haplotype in *Y. aloifolia* and *Y. gloriosa*, our findings implicate multiple origins of *Y. gloriosa* with both *Y. aloifolia* and *Y. filamentosa* acting as maternal parents. Moreover, analyses of nuclear TE abundances document overall quite similar TE landscapes across the three species, but certain families showed species-specific shifts in abundance. Using mRNA to assess current transposon activity, we find little evidence for ongoing release of transposons in the hybrid genome.

Reciprocal Parentage and Multiple Origins

Using 15–40x whole genome resequencing data, chloroplast assemblies for 38 individuals of *Yucca* across three species provided robust re-assessment of the history of this hybrid system. The presence of three separate clades containing *Y. gloriosa* (**Figure 2**) strongly suggests that not only can *Y. aloifolia* act as the maternal parent in the cross, as previously suggested, but that a reciprocal cross with *Y. filamentosa* as the maternal parent was viable enough to produce at least one extant



lineage in *Y. gloriosa*. While *Y. filamentosa* acting as the maternal parent in at least one cross is a parsimonious explanation for the data, the presence of a *Y. filamentosa* chloroplast in *Y. gloriosa* could also be due to a backcrossing event in which a *Y. gloriosa* pollen grain sired a seed on a *Y. filamentosa* individual. Such a backcross is unlikely to have happened recently. All of the genotypes of *Y. gloriosa* in this study ($n = 24$), as well as 2 and 6 of the genotypes for *Y. aloifolia* and *Y. filamentosa* from this study, respectively, have been phenotyped extensively for photosynthesis related traits (Heyduk et al., 2020), and a recent backcrossed hybrid would be expected to have photosynthetic physiology more similar to *Y. filamentosa* than *Y. aloifolia*,

as the parents are strongly divergent in whether they use C_3 photosynthesis or CAM, respectively. However, the genotype of *Y. gloriosa* with the *Y. filamentosa* chloroplast haplotype (YG16) has strong signatures of CAM, including nocturnal CO_2 uptake as well as acid accumulation, traits which are diagnostic of the CAM phenotype displayed by *Y. aloifolia* (Heyduk et al., 2020). Additionally, the three species are very easy to distinguish in the field by leaf morphology: *Y. filamentosa* has filamentous leaf margins, *Y. aloifolia* has serrated leaf margins, and *Y. gloriosa* has smooth leaf margins. However these observations cannot rule out a more ancient backcrossing event, in which an original *Y. filamentosa* x *Y. gloriosa* cross's progeny thereafter crossed only

within *Y. gloriosa*, which over time would largely dampen the addition of the *Y. filamentosa* nuclear genome but the chloroplast haplotype would remain.

The two clades of *Y. gloriosa* individuals that group with *Y. aloifolia* further support the inference that *Y. gloriosa* is derived from multiple hybridization events. However, as with the one instance of a *Y. filamentosa* chloroplast in *Y. gloriosa*, it is difficult to rule out recent backcrossing as the source of this observation (though leaf margins of all *Y. gloriosa* individuals sampled here had smooth margins that are diagnostic of this species in the wild). Earlier literature suggests that *Y. aloifolia* was introduced into the Southeastern United States ~500 years ago by European colonists, who likely brought the plant from the Caribbean or Central America (Trelease, 1893; Groman and Pellmyr, 2000; Pellmyr, 2003). Within that time frame, however, the age of the hybridization events remains unknown. Additional analysis of re-sequencing data will assist in determining the number and timing of putative hybridization events. For example, the length of parental haplotype segments in a hybrid genome is related to the degree of recombination across the hybrid genome; short haplotype blocks would indicate a greater degree of recombination and, therefore, an older hybridization event. On the other hand, longer intact parental haplotype blocks in the hybrid may point to more recent hybridization. Moreover, the length of these haplotype blocks will vary between individuals, and may point to a mixture of both older and younger hybridization events within *Y. gloriosa*.

Previous work on the three *Yucca* species suggested that all *Y. aloifolia* and *Y. gloriosa* individuals shared a single chloroplast haplotype (Rentsch and Leebens-Mack, 2012). Comparisons across the entire chloroplast genome show that four nucleotide differences separated the two clades of *Y. aloifolia* and *Y. gloriosa* individuals. Over 400 genetic changes separate the *Y. filamentosa* and YG16 haplotypes from all *Y. aloifolia* and the remaining *Y. gloriosa* haplotypes. In agreement with the previous work, this study documents low plastid genetic diversity within *Y. aloifolia* and most *Y. gloriosa* samples. *Yucca aloifolia* is introduced into the Southeastern United States and likely suffered a bottleneck, resulting in lower overall diversity. The current sample of *Y. gloriosa* individuals identified one individual with a *Y. filamentosa*-derived haplotype. Additionally, this analysis identified seven discrete haplotypes within *Y. filamentosa*, which parallels the greater number of alleles per locus in *Y. filamentosa* suggested by previous work (Rentsch and Leebens-Mack, 2012).

Any attempt at describing the frequency of hybrid formation will be largely affected by the number of individuals in the germplasm collection. The original collection area spanned a large portion of the Southeastern United States in order to capture a significant amount of genetic diversity within the genus. Collections of *Y. gloriosa* likely represent many of the extant populations, but the ranges of both *Y. aloifolia* and *Y. filamentosa* are much larger than sampled here. As a result, any interpretation of geographic patterns to the chloroplast phylogeny or haplotype network are hampered by relatively low sampling of the parental genetic diversity. For example, the single *Y. gloriosa* individual found with a *Y. filamentosa* chloroplast (YG16) was collected in South Carolina, while *Y. filamentosa* individuals with the most

similar haplotypes were collected in Delaware, North Carolina, and South Carolina. This haplotype grouping is clearly not geographically localized to one portion of the Atlantic coast and could be the result of missing genetic diversity in our analysis. Additionally, the Southeastern United States coastline experiences hurricanes and/or tropical storms on nearly an annual basis. Such storms have the potential to both disperse genets as well as eradicate entire populations and could make geographic interpretation of extant diversity difficult.

Transposable Element Abundance and Amplification

Genome resequencing provides a relatively unbiased sampling of the genome, allowing us to estimate the genomic fraction composed of transposable elements. Among sequenced plant genomes, transposable element contribution to genome size ranges from 14% in *Eragrostis tef* to 85% in *Zea mays* (Wendel et al., 2016). While all three *Yucca* species described in this work fall within the described range, the three species varied in the total amount of repetitive DNA with *Y. filamentosa* having significantly less repetitive DNA than *Y. aloifolia* and *Y. gloriosa* (62% vs. 65/66%). However, variation in abundance of particular repeat superfamilies does suggest superfamily-specific changes between the three species. *Copia* elements, the second most abundant superfamily of repeat in all three species, were more abundant in *Y. gloriosa* relative to both parents, suggesting an amplification of this superfamily post-hybridization. While Class 2 elements represent a relatively small proportion of *Yucca* genomes, *Helitrons* were found more often in *Y. filamentosa* compared to either *Y. aloifolia* or *Y. gloriosa*. *Helitrons* are capable of generating a tremendous amount of structural novelty, including the ability to capture and re-distribute pieces of genes (Yang and Bennetzen, 2009). As genomes become available for these species, it will be possible to analyze the extent to which all types of transposable elements have facilitated structural rearrangements and have affected expression of neighboring genes.

Previous work in various hybrid systems has shown incredible changes to the genomes post-hybridization. In a wallaby x kangaroo cross, reduced methylation of the genome resulted in the proliferation of a novel transposable element that caused significant structural changes to the chromosomes (O'Neill et al., 1998). Interspecific hybrids in *Drosophila* had an increase in transposable element mobilization relative to parental species (Vela et al., 2014). Three independent homoploid hybrids in *Helianthus* all show increased genome size due to expansion of repetitive elements, particularly in *Ty3/gypsy-like* LTR elements (Ungerer et al., 2006, 2009). In *Yucca*, however, there seems to be little indication that transposable elements were released from silencing mechanisms and proliferated in the hybrid *Y. gloriosa*. Instead, *Y. gloriosa* shows similar abundance of transposable elements relative to its progenitor species, though with a notable increase in *Copia* elements in the hybrid (Figure 4). Extant genotypes of *Y. gloriosa* have little in the way of increased repeat expression (Figure 6); whether this means no genomic shock initially happened upon hybridization, or that the

genome has had sufficient time to stabilize repetitive elements, remains unclear.

Finally, the three *Yucca* species provide an excellent system within which to describe the role of repetitive content on novel phenotypic evolution and adaptation. *Yucca gloriosa* has been studied extensively for its intermediate photosynthetic phenotype (Heyduk et al., 2016, 2019). When well-watered, the majority of carbon fixation happens during the day through the C₃ cycle, although low levels of CAM activity are present. When drought stress, *Y. gloriosa* can switch to predominantly CAM photosynthesis, but the degree to which individual genotypes do so varies. The hybrid's photosynthetic phenotype is novel, in that neither parent displays CAM induction upon drought stress, nor the ability to switch from primarily C₃ carbon fixation to primarily CAM. On first glance, negligible differences in repeat content and activity in *Y. gloriosa* relative to its parents suggest that repetitive content is unlikely to underlie the novel photosynthetic phenotype in the hybrid. However, here we only assessed overall abundance and activity in extant individuals; location of repeats in the hybrid relative to the parental species, as well as older repetitive content bursts, still have the potential to create transgressive and novel phenotypes in the hybrid. Repetitive elements can alter gene expression and gene networks by inserting into regulatory regions (Kunaroo et al., 2010; Wang et al., 2013), can interfere with alternative splicing (Leprince et al., 2001; Li et al., 2014), and can be a general source of genomic variation and rapid evolution (González et al., 2010; Schrader et al., 2014). Moreover, transposable element activity can increase in response to environmental stressors (Makarevitch et al., 2015) and can play a role in forming stress-induced regulatory networks (Naito et al., 2009). Whether transposable elements are responsible for *Y. gloriosa*'s ability to upregulate CAM photosynthesis under drought stress remains to be tested.

CONCLUSION

Since the chloroplast phylogeny and haplotype network imply multiple hybridization events contributing to the origin of *Y. gloriosa*, new hypotheses regarding the repeatability of transposon accumulation can now be tested. For example, since YG16 appears to most likely be derived from a distinct hybridization event relative to other *Y. gloriosa* genotypes, we can assess whether the genomic organization of its transposable elements is vastly different from the major clade of *Y. gloriosa* genotypes grouping with *Y. aloifolia* (Figure 2). Integrating transposable element abundance and expression with other types of genomic data, including RNA-seq and bisulfite sequencing, may help us understand the potential for insertions to differentially regulate genes. The *Yucca* system is particularly powerful, in that the parental species are strongly divergent in photosynthetic pathway and the hybrid segregates for many of the same traits; this provides a framework in which to understand the role of repeats in regulating these genes in *Y. gloriosa*.

Given the massively expanding availability of whole genome sequence data, hypothesis-driven comparative analyses of genome content and structure are becoming more tractable. In

this work, reads that normally would have been filtered out were instead analyzed to address whether a hybrid species had multiple and/or reciprocal origins. Furthermore, these reads helped provide a first glance into the repetitive landscape of 40 genotypes across three related species. While whole genomes will ultimately have the greatest ability to answer many of the questions brought up in this work, the approaches used here are quicker, less expensive, and generate many hypotheses for testing at the genome level in the future.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in **Supplementary Table 1**.

AUTHOR CONTRIBUTIONS

JG prepared libraries and sequenced samples. SS annotated repetitive content in the parental genomes. EM and KH conducted all analyses and wrote the manuscript. MM optimized plastome assembly and assisted with the manuscript. JL-M and JS were integral to overall project planning and management and assisted with the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2020.573767/full#supplementary-material>

REFERENCES

- Abraham, P. E., Yin, H., Borland, A. M., Weighill, D., Lim, S. D., De Paoli, H. C., et al. (2016). Transcript, protein and metabolite temporal dynamics in the CAM plant Agave. *Nat. Plants* 2:16178.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410.
- Arnold, M. L. (1993). *Iris nelsonii* (Iridaceae): origins and genetic composition of a homoploid hybrid species. *Am. J. Bot.* 80, 577–583. doi: 10.1002/j.1537-2197.1993.tb13843.x
- Bao, Z., and Eddy, S. R. (2002). Automated de novo identification of repeat sequence families in sequenced genomes. *Genome Res.* 12, 1269–1276. doi: 10.1101/gr.88502
- Bardil, A., de Almeida, J. D., Combes, M. C., Lashermes, P., and Bertrand, B. (2011). Genomic expression dominance in the natural allopolyploid *Coffea arabica* is massively affected by growth temperature. *New Phytol.* 192, 760–774. doi: 10.1111/j.1469-8137.2011.03833.x
- Bird, K. A., VanBuren, R., Puzey, J. R., and Edger, P. P. (2018). The causes and consequences of subgenome dominance in hybrids and recent polyploids. *New Phytol.* 220, 87–93. doi: 10.1111/nph.15256
- Boisvert, S., Lavolette, F., and Corbeil, J. (2010). Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. *J. Comput. Biol.* 17, 1519–1533. doi: 10.1089/cmb.2009.0238
- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170
- Bray, N. L., Pimentel, H., Melsted, P., and Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nat. Biotechnol.* 34, 525–527. doi: 10.1038/nbt.3519
- Bredeson, J. V., Lyons, J. B., Prochnik, S. E., Wu, G. A., Ha, C. M., Edsinger-Gonzales, E., et al. (2016). Sequencing wild and cultivated cassava and related species reveals extensive interspecific hybridization and genetic diversity. *Nat. Biotechnol.* 34, 562–570. doi: 10.1038/nbt.3535
- Danilova, T. V., Akhunova, A. R., Akhunov, E. D., Friebe, B., and Gill, B. S. (2017). Major structural genomic alterations can be associated with hybrid speciation in *Aegilops markgrafii* (Triticeae). *Plant J.* 92, 317–330. doi: 10.1111/tbj.13657
- Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9:772. doi: 10.1038/nmeth.2109
- Devos, K. M., Brown, J. K. M., and Bennetzen, J. L. (2002). Genome size reduction through illegitimate recombination counteracts genome expansion in *Arabidopsis*. *Genome Res.* 12, 1075–1079. doi: 10.1101/gr.132102
- Dion-Côté, A.-M., Renaut, S., Normandeau, E., and Bernatchez, L. (2014). RNA-seq reveals transcriptomic shock involving transposable elements reactivation in hybrids of young lake whitefish species. *Mol. Biol. Evol.* 31, 1188–1199. doi: 10.1093/molbev/msu069
- Doyle, J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19, 11–15.
- Edger, P. P., Smith, R., McKain, M. R., Cooley, A. M., Vallejo-Marin, M., Yuan, Y., et al. (2017). Subgenome dominance in an interspecific hybrid, synthetic allopolyploid, and a 140-year-old naturally established neo-allopolyploid monkeyflower. *Plant Cell* 29, 2150–2167. doi: 10.1105/tpc.17.00010
- Feschotte, C., and Pritham, E. J. (2007). DNA transposons and the evolution of eukaryotic genomes. *Annu. Rev. Genet.* 41, 331–368. doi: 10.1146/annurev.genet.40.110405.090448
- Fultz, D., Choudury, S. G., and Slotkin, R. K. (2015). Silencing of active transposable elements in plants. *Curr. Opin. Plant Biol.* 27, 67–76. doi: 10.1016/j.pbi.2015.05.027
- González, J., Karasov, T. L., Messer, P. W., and Petrov, D. A. (2010). Genome-wide patterns of adaptation to temperate environments associated with transposable elements in *Drosophila*. *PLoS Genet.* 6:e1000905. doi: 10.1371/journal.pgen.1000905
- Groman, J. D., and Pellmyr, O. (2000). Rapid evolution and specialization following host colonization in a yucca moth. *J. Evol. Biol.* 13, 223–236. doi: 10.1046/j.1420-9101.2000.00159.x
- Gross, B. L., and Rieseberg, L. H. (2005). The ecological genetics of homoploid hybrid speciation. *J. Hered.* 96, 241–252. doi: 10.1093/jhered/esi026
- Hegarty, M. J., Barker, G. L., Brennan, A. C., Edwards, K. J., Abbott, R. J., and Hiscock, S. J. (2009). Extreme changes to gene expression associated with homoploid hybrid speciation. *Mol. Ecol.* 18, 877–889. doi: 10.1111/j.1365-294x.2008.04054.x
- Hersch, E. I., and Roy, B. A. (2007). Context-dependent pollinator behavior: an explanation for patterns of hybridization among three species of Indian paintbrush. *Evolution* 61, 111–124. doi: 10.1111/j.1558-5646.2007.00009.x
- Heyduk, K., Burrell, N., Lalani, F., and Leebens-Mack, J. (2016). Gas exchange and leaf anatomy of a C3-CAM hybrid, *Yucca gloriosa* (Asparagaceae). *J. Exp. Bot.* 67, 1369–1379. doi: 10.1093/jxb/erv336
- Heyduk, K., Ray, J. N., Ayyampalayam, S., Moledina, N., Borland, A., Harding, S. A., et al. (2019). Shared expression of Crassulacean acid metabolism (CAM) genes predates the origin of CAM in the genus *Yucca*. *J. Exp. Bot.* 70, 6597–6609. doi: 10.1093/jxb/erz105
- Heyduk, K., Ray, J. N., and Leebens-Mack, J. (2020). Leaf anatomy is not correlated to CAM function in a C3+CAM hybrid species, *Yucca gloriosa*. *Ann. Bot.* 2020:mcaa036. doi: 10.1093/aob/mcaa036
- Hollister, J. D., Smith, L. M., Guo, Y.-L., Ott, F., Weigel, D., and Gaut, B. S. (2011). Transposable elements and small RNAs contribute to gene expression divergence between *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2322–2327. doi: 10.1073/pnas.1018222108
- Johnston, J. A., Grise, D. J., Donovan, L. A., and Arnold, M. L. (2001). Environment-dependent performance and fitness of *Iris brevicaulis*, *I. fulva* (Iridaceae), and hybrids. *Am. J. Bot.* 88, 933–938. doi: 10.2307/2657046
- Karrenberg, S., Edelist, C., Lexer, C., and Rieseberg, L. (2006). Response to salinity in the homoploid hybrid species *Helianthus paradoxus* and its progenitors *H. annuus* and *H. petiolaris*. *New Phytol.* 170, 615–629. doi: 10.1111/j.1469-8137.2006.01687.x
- Katoh, K., and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010
- Kunarse, G., Chia, N.-Y., Jeyakani, J., Hwang, C., Lu, X., Chan, Y.-S., et al. (2010). Transposable elements have rewired the core regulatory network of human embryonic stem cells. *Nat. Genet.* 42, 631–634. doi: 10.1038/ng.600
- Lai, Z., Gross, B. L., Zou, Y. I., Andrews, J., and Rieseberg, L. H. (2006). Microarray analysis reveals differential gene expression in hybrid sunflower species. *Mol. Ecol.* 15, 1213–1227. doi: 10.1111/j.1365-294x.2006.02775.x
- Lai, Z., Nakazato, T., Salmasso, M., Burke, J. M., Tang, S., Knapp, S. J., et al. (2005). Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. *Genetics* 171, 291–303. doi: 10.1534/genetics.105.042242
- Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi: 10.1038/nmeth.1923
- Leebens-Mack, J., and Milligan, B. (1998). Pollination biology in hybridizing *Baptisia* (Fabaceae) populations. *Am. J. Bot.* 85:500. doi: 10.2307/2446433
- Leebens-Mack, J., and Pellmyr, O. (2004). Patterns of genetic structure among populations of an oligophagous pollinating yucca moth (*Tegeticula yuccasella*). *J. Hered.* 95, 127–135. doi: 10.1093/jhered/esh025
- Leigh, J. W., and Bryant, D. (2015). popart : full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6, 1110–1116. doi: 10.1111/2041-210x.12410
- Leprince, A. S., Grandbastien, M. A., and Meyer, C. (2001). Retrotransposons of the Tnt1B family are mobile in *Nicotiana glauca* and can induce alternative splicing of the host gene upon insertion. *Plant Mol. Biol.* 47, 533–541.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079. doi: 10.1093/bioinformatics/btp352
- Li, Q., Xiao, G., and Zhu, Y.-X. (2014). Single-nucleotide resolution mapping of the *Gossypium raimondii* transcriptome reveals a new mechanism for alternative splicing of introns. *Mol. Plant* 7, 829–840. doi: 10.1093/mp/sst175
- Lisch, D. (2009). Epigenetic regulation of transposable elements in plants. *Annu. Rev. Plant Biol.* 60, 43–66. doi: 10.1146/annurev.arplant.59.032607.092744
- Makarevitch, I., Waters, A. J., West, P. T., Stitzer, M., Hirsch, C. N., Ross-Ibarra, J., et al. (2015). Transposable elements contribute to activation of maize genes in

- response to abiotic stress. *PLoS Genet.* 11:e1004915. doi: 10.1371/journal.pgen.1004915
- McClintock, B. (1984). The significance of responses of the genome to challenge. *Science* 226, 792–801. doi: 10.1126/science.15739260
- McKain, M. R., McNeal, J. R., Kellar, P. R., Eguiarte, L. E., Pires, J. C., and Leebens-Mack, J. (2016). Timing of rapid diversification and convergent origins of active pollination within *Agavoideae* (Asparagaceae). *Am. J. Bot.* 103, 1717–1729. doi: 10.3732/ajb.1600198
- Miller, M. A., Pfeiffer, W., and Schwartz, T. (2010). “Creating the CIPRES Science Gateway for inference of large phylogenetic trees,” in *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, LA, 1–8.
- Naito, K., Zhang, F., Tsukiyama, T., Saito, H., Hancock, C. N., Richardson, A. O., et al. (2009). Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. *Nature* 461, 1130–1134. doi: 10.1038/nature08479
- O’Neill, R. J., O’Neill, M. J., and Graves, J. A. (1998). Undermethylation associated with retroelement activation and chromosome remodeling in an interspecific mammalian hybrid. *Nature* 393, 68–72. doi: 10.1038/29985
- Parisod, C., Salmon, A., Zerjal, T., Tenaillon, M., Grandbastien, M.-A., and Ainouche, M. (2009). Rapid structural and epigenetic reorganization near transposable elements in hybrid and allopolyploid genomes in *Spartina*. *New Phytol.* 184, 1003–1015. doi: 10.1111/j.1469-8137.2009.03029.x
- Pellmyr, O. (1999). Systematic revision of the yucca moths in the *Tegeticula yuccasella* complex (Lepidoptera: Prodoxidae) north of Mexico. *Syst. Entomol.* 24, 243–271. doi: 10.1046/j.1365-3113.1999.00079.x
- Pellmyr, O. (2003). *Yuccas, Yucca moths, and coevolution: a review*. *Ann. Mo. Bot. Gard.* 90, 35–55. doi: 10.2307/3298524
- Price, A. L., Jones, N. C., and Pevzner, P. A. (2005). De novo identification of repeat families in large genomes. *Bioinformatics* 21(Suppl. 1), i351–i358.
- Quinlan, A. R., and Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics* 26, 841–842. doi: 10.1093/bioinformatics/btq033
- R Core Team (2019). *R: A Language and Environment for Statistical Computing*. Vienna: R foundation for statistical computing.
- Rapp, R. A., Udall, J. A., and Wendel, J. F. (2009). Genomic expression dominance in allopolyploids. *BMC Biol.* 7:18. doi: 10.1186/1741-7007-7-18
- Renaut, S., Rowe, H. C., Ungerer, M. C., and Rieseberg, L. H. (2014). Genomics of homoploid hybrid speciation: diversity and transcriptional activity of long terminal repeat retrotransposons in hybrid sunflowers. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:20130345. doi: 10.1098/rstb.2013.0345
- Rentsch, J. D., and Leebens-Mack, J. (2012). Homoploid hybrid origin of *Yucca gloriosa*: intersectional hybrid speciation in *Yucca* (Agavoideae, Asparagaceae). *Ecol. Evol.* 2, 2213–2222. doi: 10.1002/ece3.328
- Rentsch, J. D., and Leebens-Mack, J. (2014). *Yucca aloifolia* (Asparagaceae) opts out of an obligate pollination mutualism. *Am. J. Bot.* 101, 2062–2067. doi: 10.3732/ajb.1400351
- Rieseberg, L. H. (1991). Homoploid reticulate evolution in *Helianthus* (asteraceae): evidence from ribosomal genes. *Am. J. Bot.* 78, 1218–1237. doi: 10.1002/j.1537-2197.1991.tb11415.x
- Rieseberg, L. H. (1997). Hybrid origins of plant species. *Annu. Rev. Ecol. Syst.* 28, 359–389. doi: 10.1146/annurev.ecolsys.28.1.359
- Rieseberg, L. H., Raymond, O., Rosenthal, D. M., Lai, Z., Livingstone, K., Nakazato, T., et al. (2003). Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301, 1211–1216. doi: 10.1126/science.1086949
- Rieseberg, L. H., Van Fossen, C., and Desrochers, A. M. (1995). Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* 375, 313–316. doi: 10.1038/375313a0
- Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140. doi: 10.1093/bioinformatics/btp616
- Robinson, M. D., and Oshlack, A. (2010). A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol.* 11:R25.
- Salmon, A., Ainouche, M. L., and Wendel, J. F. (2005). Genetic and epigenetic consequences of recent hybridization and polyploidy in *Spartina* (Poaceae). *Mol. Ecol.* 14, 1163–1175. doi: 10.1111/j.1365-294x.2005.02488.x
- Sapir, Y., Moody, M. L., Brouillette, L. C., Donovan, L. A., and Rieseberg, L. H. (2007). Patterns of genetic diversity and candidate genes for ecological divergence in a homoploid hybrid sunflower, *Helianthus anomalus*. *Mol. Ecol.* 16, 5017–5029. doi: 10.1111/j.1365-294x.2007.03557.x
- Schnable, J. C., Springer, N. M., and Freeling, M. (2011). Differentiation of the maize subgenomes by genome dominance and both ancient and ongoing gene loss. *Proc. Natl. Acad. Sci. U.S.A.* 108, 4069–4074. doi: 10.1073/pnas.1101368108
- Schrader, L., Kim, J. W., Ence, D., Zimin, A., Klein, A., Wyschetzki, K., et al. (2014). Transposable element islands facilitate adaptation to novel environments in an invasive species. *Nat. Commun.* 5:5495.
- Smit, A. F. A., and Hubley, R. (2008). *RepeatModeler Open-1.0*.
- Soltis, P. S., and Soltis, D. E. (2009). The role of hybridization in plant speciation. *Annu. Rev. Plant Biol.* 60, 561–588. doi: 10.1146/annurev.arplant.043008.092039
- Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690. doi: 10.1093/bioinformatics/btl446
- Staton, S. E., Bakken, B. H., Blackman, B. K., Chapman, M. A., Kane, N. C., Tang, S., et al. (2012). The sunflower (*Helianthus annuus* L.) genome reflects a recent history of biased accumulation of transposable elements. *Plant J.* 72, 142–153. doi: 10.1111/j.1365-313x.2012.05072.x
- Staton, S. E., and Burke, J. M. (2015). Transposome: a toolkit for annotation of transposable element families from unassembled sequence reads. *Bioinformatics* 31, 1827–1829. doi: 10.1093/bioinformatics/btv059
- Staton, S. E., and Ungerer, M. C. (2009). The genomic organization of Ty3/gypsy-like retrotransposons in *Helianthus* (Asteraceae) homoploid hybrid species. *Am. J. Bot.* 96, 1646–1655. doi: 10.3732/ajb.080337
- Štorchová, H., Hrdličková, R., Chrtěk, J., Tetera, M., Fitze, D., and Fehrer, J. (2000). An improved method of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. *Taxon* 49, 79–84. doi: 10.2307/1223934
- Studer, A., Zhao, Q., Ross-Ibarra, J., and Doebley, J. (2011). Identification of a functional transposon insertion in the maize domestication gene *tb1*. *Nat. Genet.* 43, 1160–1163. doi: 10.1038/ng.942
- Sun, C., Ma, Z., Zhang, Z., Sun, G., and Dai, Z. (2018). Factors Influencing Cross Barriers in Interspecific Hybridizations of Water Lily. *J. Am. Soc. Hortic. Sci.* 143, 130–135. doi: 10.21273/jashs04302-17
- Tenaillon, M. I., Hufford, M. B., Gaut, B. S., and Ross-Ibarra, J. (2011). Genome size and transposable element content as determined by high-throughput sequencing in maize and *Zea luxurians*. *Genome Biol. Evol.* 3, 219–229. doi: 10.1093/gbe/evr008
- Thórrson, A. T., Salmela, E., and Anamthawat-Jónsson, K. (2001). Morphological, cytogenetic, and molecular evidence for introgressive hybridization in birch. *J. Hered.* 92, 404–408. doi: 10.1093/jhered/92.5.404
- Trelease, W. (1893). Further studies of yuccas and their pollination. *Missouri Bot. Garden Annu. Rep.* 1893, 181–226. doi: 10.2307/2992178
- Trelease, W. (1902). The yuccaeae. *Missouri Bot. Garden Annu. Rep.* 1902, 27–133. doi: 10.2307/2400121
- Ungerer, M. C., Strakosh, S. C., and Stimpson, K. M. (2009). Proliferation of Ty3/gypsy-like retrotransposons in hybrid sunflower taxa inferred from phylogenetic data. *BMC Biol.* 7:40. doi: 10.1186/1741-7007-7-40
- Ungerer, M. C., Strakosh, S. C., and Zhen, Y. (2006). Genome expansion in three hybrid sunflower species is associated with retrotransposon proliferation. *Curr. Biol.* 16, R872–R873.
- Vela, D., Fontdevila, A., Vieira, C., and García Guerreiro, M. P. (2014). A genome-wide survey of genetic instability by transposition in *Drosophila* hybrids. *PLoS One* 9:e88992. doi: 10.1371/journal.pone.0088992
- Venables, W. N., and Ripley, B. D. (2013). *Modern Applied Statistics with S-PLUS*. Cham: Springer.
- Wang, H., McArthur, E. D., Sanderson, S. C., Graham, J. H., and Freeman, D. C. (1997). Narrow hybrid zone between two species of Big Sagebrush (*Artemisia tridentata*: Asteraceae). IV. Reciprocal transplant experiments. *Evolution* 51, 95–102. doi: 10.2307/2410963
- Wang, X., Weigel, D., and Smith, L. M. (2013). Transposon variants and their effects on gene expression in *Arabidopsis*. *PLoS Genet.* 9:e1003255. doi: 10.1371/journal.pgen.1003255
- Welch, M. E., and Rieseberg, L. H. (2002). Habitat divergence between a homoploid hybrid sunflower species, *Helianthus paradoxus* (Asteraceae),

- and its progenitors. *Am. J. Bot.* 89, 472–478. doi: 10.3732/ajb.89.3.472
- Wendel, J. F., Jackson, S. A., Meyers, B. C., and Wing, R. A. (2016). Evolution of plant genome architecture. *Genome Biol.* 17:37.
- Wessler, S. R., Bureau, T. E., and White, S. E. (1995). LTR-retrotransposons and MITEs: important players in the evolution of plant genomes. *Curr. Opin. Genet. Dev.* 5, 814–821. doi: 10.1016/0959-437x(95)80016-x
- Wicker, T., Sabot, F., Hua-Van, A., Bennetzen, J. L., Capy, P., Chalhoub, B., et al. (2007). A unified classification system for eukaryotic transposable elements. *Nat. Rev. Genet.* 8, 973–982. doi: 10.1038/nrg2165
- Xiao, H., Jiang, N., Schaffner, E., Stockinger, E. J., and van der Knaap, E. (2008). A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* 319, 1527–1530. doi: 10.1126/science.1153040
- Xu, Y., Zhong, L., Wu, X., Fang, X., and Wang, J. (2009). Rapid alterations of gene expression and cytosine methylation in newly synthesized *Brassica napus* allopolyploids. *Planta* 229, 471–483. doi: 10.1007/s00425-008-0844-8
- Yang, L., and Bennetzen, J. L. (2009). Distribution, diversity, evolution, and survival of Helitrons in the maize genome. *Proc. Natl. Acad. Sci. U.S.A.* 106, 19922–19927. doi: 10.1073/pnas.0908008106
- Yoo, M.-J., Szadkowski, E., and Wendel, J. F. (2013). Homoeolog expression bias and expression level dominance in allopolyploid cotton. *Heredity* 110, 171–180. doi: 10.1038/hdy.2012.94

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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