

# 1 Extensive genome evolution distinguishes maize within a 2 stable tribe of grasses

---

3  
4 Michelle C. Stitzer<sup>1</sup>, Arun S. Seetharam<sup>2</sup>, Armin Scheben<sup>3</sup>, Sheng-Kai Hsu<sup>1</sup>, Aimee J. Schulz<sup>4</sup>,  
5 Taylor M. AuBuchon-Elder<sup>5</sup>, Mohamed El-Walid<sup>4</sup>, Taylor H. Ferebee<sup>6</sup>, Charles O. Hale<sup>4</sup>, Thuy La<sup>1</sup>,  
6 Zong-Yan Liu<sup>4</sup>, Sarah J. McMorrow<sup>1</sup>, Patrick Minx<sup>5</sup>, Alyssa R. Phillips<sup>7</sup>, Michael L. Syring<sup>2</sup>, Travis  
7 Wrightsman<sup>4</sup>, Jingjing Zhai<sup>1</sup>, Rémy Pasquet<sup>8</sup>, Christine A. McAllister<sup>9</sup>, Simon T. Malcomber<sup>10</sup>,  
8 Paveena Traiperm<sup>11</sup>, Daniel J. Layton<sup>12</sup>, Jinshun Zhong<sup>13</sup>, Denise E. Costich<sup>1</sup>, R. Kelly Dawe<sup>14</sup>,  
9 Kevin Fengler<sup>15</sup>, Charlotte Harris<sup>15</sup>, Zach Irelan<sup>15</sup>, Victor Llaca<sup>15</sup>, Praveena Parakkal<sup>15</sup>, Gina  
10 Zastrow-Hayes<sup>15</sup>, Margaret R. Woodhouse<sup>16</sup>, Ethalinda K. Cannon<sup>16</sup>, John L. Portwood II<sup>16</sup>,  
11 Carson M. Andorf<sup>16</sup>, Patrice S. Albert<sup>17</sup>, James A. Birchler<sup>17</sup>, Adam Siepel<sup>3</sup>, Jeffrey Ross-Ibarra<sup>7,18</sup>,  
12 M. Cinta Romay<sup>1</sup>, Elizabeth A. Kellogg<sup>5</sup>, Edward S. Buckler<sup>1,19</sup>, Matthew B. Hufford<sup>2</sup>

13  
14

15 <sup>1</sup> Institute for Genomic Diversity, Cornell University, Ithaca, NY 14850 USA

16 <sup>2</sup> Ecology, Evolution, and Organismal Biology, Iowa State University, Ames IA 50011 USA

17 <sup>3</sup> Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY  
18 11724 USA

19 <sup>4</sup> Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14850 USA

20 <sup>5</sup> Donald Danforth Plant Science Center, St. Louis, Missouri 63132, USA

21 <sup>6</sup> Department of Computational Biology, Cornell University, Ithaca, NY 14850 USA

22 <sup>7</sup> Department of Evolution and Ecology and Center for Population Biology, University of  
23 California, Davis, Davis CA 95616 USA

24 <sup>8</sup> DIADE, IRD, CIRAD, University of Montpellier, Montpellier, France

25 <sup>9</sup> Principia College, Elsah, IL 62028 USA

26 <sup>10</sup> National Science Foundation, Alexandria, VA 22314 USA

27 <sup>11</sup> Department of Plant Science, Faculty of Science, Mahidol University, Ratchathewi, Bangkok,  
28 Thailand

29 <sup>12</sup> Indiana University, Department of Biology, Bloomington, IN 47405 USA

30 <sup>13</sup> South China Agricultural University, Guangzhou, Guangdong, 510642 China

31 <sup>14</sup> Department of Genetics, University of Georgia, Athens, GA 30602 USA

32 <sup>15</sup> Corteva Agriscience, Johnson, IA 50131 USA

33 <sup>16</sup> USDA-ARS, Corn Insects and Crop Genetics Research Unit, Ames, IA 50011 USA

34 <sup>17</sup> Division of Biological Sciences, University of Missouri, Columbia MO 65211 USA

35 <sup>18</sup> Genome Center, University of California, Davis, Davis, CA 95616 USA

36 <sup>19</sup> USDA-ARS, Ithaca, NY 14850 USA

37  
38

## 39 Abstract

---

40  
41 Over the last 20 million years, the Andropogoneae tribe of grasses has evolved to  
42 dominate 17% of global land area. Domestication of these grasses in the last 10,000 years has  
43 yielded our most productive crops, including maize, sugarcane, and sorghum. The majority of  
44 Andropogoneae species, including maize, show a history of polyploidy – a condition that, while  
45 offering the evolutionary advantage of multiple gene copies, poses challenges to basic cellular  
46 processes, gene expression, and epigenetic regulation. Genomic studies of polyploidy have  
47 been limited by sparse sampling of taxa in groups with multiple polyploidy events. Here, we  
48 present 33 genome assemblies from 27 species, including chromosome-scale assemblies of  
49 maize relatives *Zea* and *Tripsacum*. In maize, the after-effects of polyploidy have been widely  
50 studied, showing reduced chromosome number, biased fractionation of duplicate genes, and  
51 transposable element (TE) expansions. While we observe these patterns within the genus *Zea*,  
52 12 other polyploidy events deviate significantly. Those tetraploids and hexaploids retain  
53 elevated chromosome number, maintain nearly complete complements of duplicate genes, and  
54 have only stochastic TE amplifications. These genomes reveal variable outcomes of polyploidy,  
55 challenging simple predictions and providing a foundation for understanding its evolutionary  
56 implications in an ecologically and economically important clade.

## 57 58 Introduction

---

59 Andropogoneae grasses have been integral to the origins and spread of grasslands,  
60 relevant to human culture and agriculture for thousands of years, and are of growing  
61 importance for their ability to rebalance the carbon cycle. These 1,200 species of warm-season  
62 grasses dominate ecosystems in Africa, North and South America, and portions of South and  
63 Southeast Asia (Gibson, 2009; Kellogg, 2015). The ancestor of these grasses evolved the highly  
64 efficient C4 photosynthesis (Bianconi et al., 2020), and all are adapted for low or variable CO<sub>2</sub>  
65 abundance, meaning these species are nitrogen and water use efficient (Ghannoum et al.,  
66 2011; Morison & Gifford, 1983; Rawson et al., 1977). Today, these grasses include the largest  
67 production crops maize and sugarcane, drought resistant sorghum, the bioenergy crop  
68 *Miscanthus*, and numerous forage grasses. Altogether, Andropogoneae grasses cover 17% of  
69 global land (Lehmann et al., 2019).

70 Allopolyploidy has been a major force in the evolution of Andropogoneae grasses, with  
71 at least ⅓ of all speciation events associated with polyploidy (Estep et al., 2014). Newly formed

72 polyploids often face meiotic abnormalities and establishment challenges (Ramsey & Schemske,  
73 2002), and genome doubling can induce epigenetic instability (Comai, 2005). Considering these  
74 challenges, it is surprising there are so many polyploids, not only in the Andropogoneae, but  
75 across the plant kingdom (Alix et al., 2017). Explanations for their prevalence range from  
76 ecological novelty of polyploids, allowing them to exploit niches or stressful environments  
77 unavailable to their diploid progenitors (Van de Peer et al., 2017), to the increased fitness and  
78 adaptive potential arising from the permanent hybridity of polyploids (Roose & Gottlieb, 1976;  
79 Stebbins, 1959). Over longer time scales, polyploids frequently revert to a diploid-like state  
80 (Baduel et al., 2018; Comai, 2005; Leitch & Leitch, 2008; Otto, 2007; J. F. Wendel, 2000, 2015),  
81 via three oft-cited mechanisms - chromosomal reduction, gene loss via fractionation, and  
82 transposable element amplification.

83 Despite these general patterns, there are major gaps in our understanding of even the  
84 best known polyploid systems (Soltis et al., 2016). Mounting evidence suggests that at least  
85 some polyploid lineages undergo minimal genome evolution (cotton, Wendel & Cronn, 2003,  
86 bamboo, Ma et al., 2024; *Arabidopsis suecica*, Burns et al., 2021), and other plant lineages  
87 rarely produce polyploids (gymnosperms, Ickert-Bond et al., 2020), illustrating exceptions to the  
88 conventional models. Here, we present genome assemblies of 33 Andropogoneae individuals  
89 from 27 species, capturing 14 independent polyploid formation events. Of these, twelve have  
90 little evidence of large-scale genomic reorganization, show unpredictable TE dynamics, and  
91 retain multiples of the base chromosome number and most genes. In contrast, the lineage  
92 leading to *Zea* exhibits notable changes, with expanded TEs, and only retaining a subset of  
93 progenitor genes in a radically rearranged karyotype.

94

## 95 **Results and Discussion**

---

### 96 **Genome assemblies of Andropogoneae**

97

98 We generated highly contiguous assemblies of 33 members of Andropogoneae,  
99 representing 27 species (Supplemental Table S1). Sequencing technologies evolved throughout  
100 our project, so while most individuals were sequenced with PacBio HiFi (27 plants), 5 plants  
101 were sequenced with PacBio Continuous Long Read (CLR) sequencing, and one with Oxford

102 Nanopore Technology (ONT) (Table S2). To further improve contiguity, 24 assemblies were  
103 scaffolded using BioNano optical maps, HiC, or genetic maps (Table S2). Most samples come  
104 from outbred accessions, and many come from species with multiple ploidy levels and mixed-  
105 ploidy populations.

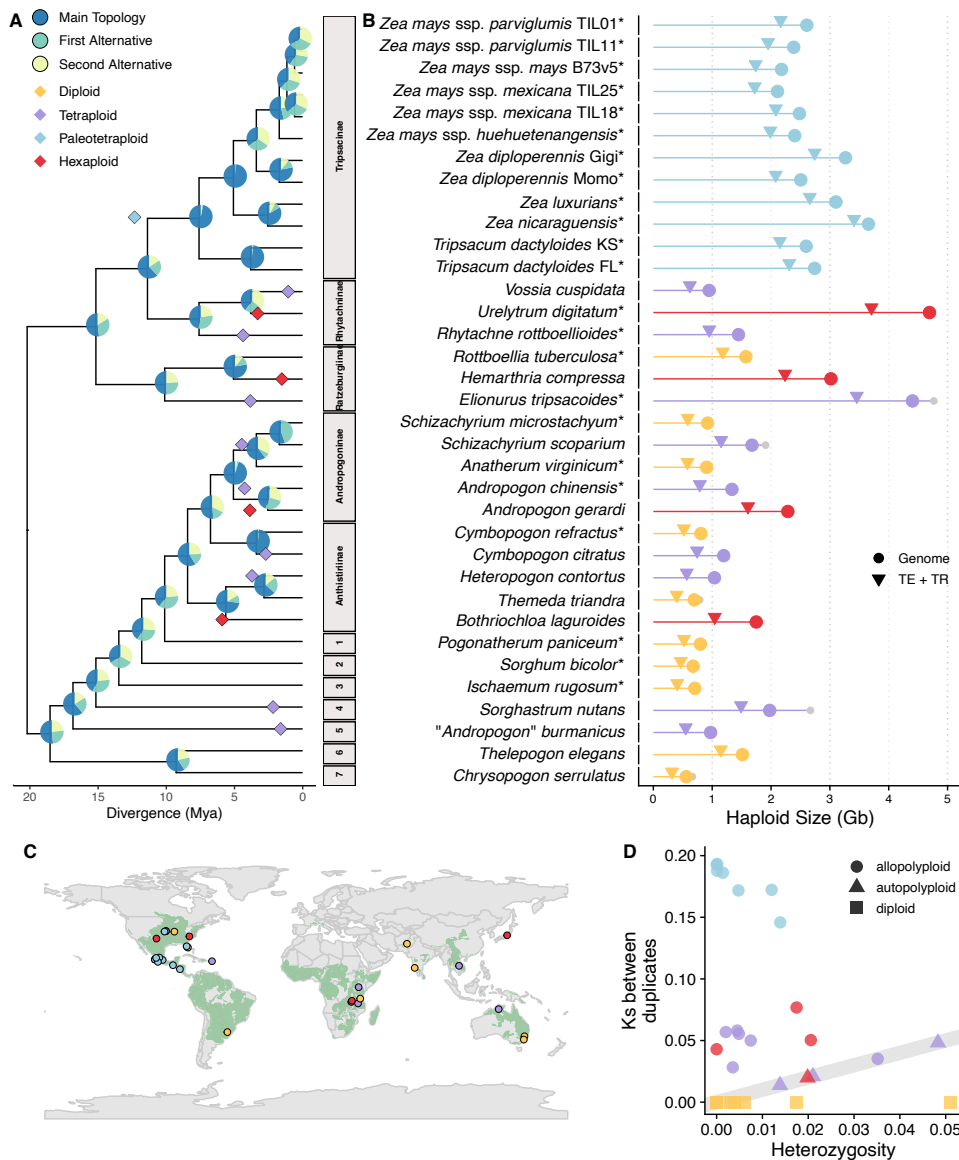
106 Our assemblies include nine chromosome-scale assemblies of all diploid ( $2n=20$ )  
107 teosinte species and subspecies within *Zea*, as well as two individuals of *Tripsacum dactyloides*  
108 ( $2n=36$ ), together representing the subtribe Tripsacinae. The 22 additional species sequenced  
109 within the tribe Andropogoneae (Supplemental Table S1) represent major clades in the  
110 phylogeny (Figure 1A) and the global distribution (Figure 1C). We observed a broad range of  
111 haploid assembly sizes, from 663 Mb to 4,767 Mb (Figure 1B; Supplemental Table S3), and  
112 found assembly size to nearly perfectly mirror genome size estimated from flow cytometry  
113 (Pearson's correlation,  $r=0.916$ ,  $p=5.75e-09$ ) (Supplemental Figure S1). The presence of  
114 telomere repeats on both terminal ends of chromosome-length contigs in 22 species further  
115 supports assembly completeness, as does contig N50 (median 10.2 Mb; range 152 kb-189 Mb)  
116 and scaffold N50 (median 72.0 Mb; range 13-198 Mb) (Supplemental Table S2). This contiguity  
117 is high in spite of high levels of repetitive transposable element (TE) and tandem repeat  
118 sequence, ranging from 54.7-93.3% of assembled sequence (Figure 1B). We generated gene  
119 model annotations for each assembly using Helixer (Stiehler et al., 2021), and predicted  
120 transcriptomes show a high level of completeness with most (median 98.54, range 95.7%-  
121 99.8%) Poales BUSCO genes identified (Supplemental Table S2).

122 Combined with publicly available assemblies of *Sorghum bicolor* and *Zea mays* subsp.  
123 *mays*, these assemblies offer a dense sample of recent (ca. 0.650 million years of evolution;  
124 Tripsacinae (Chen et al., 2022)) and a broad sample of deeper history (ca. 17.5 million years;  
125 Andropogoneae (Welker et al., 2020)).

## 126 **Relationships between Andropogoneae species**

127 To reconstruct phylogenetic relationships of these genomes, we used gene trees  
128 constructed from 7,725 syntenic gene anchors (details provided below) to generate a species  
129 tree (Fig 1A). We find pervasive conflict across gene tree topologies, with most bipartitions

130 along the internal branches of the radiation supported by alternative topologies (Figure 1A).  
 131 This conflict has been noted in previous studies of nuclear and chloroplast markers (Estep et al.,  
 132 2014; Grass Phylogeny Working Group III, 2024; Welker et al., 2020). The success of these  
 133 radiations and large effective population sizes likely contribute to these conflicting topologies,  
 134 as does extensive allopolyploidy in the clade (Estep et al., 2014). Extensive diversification of  
 135 these species occurred in the late Miocene 12-20 million years ago (Estep et al., 2014) after the  
 136 origin of C4 photosynthesis (Bianconi et al., 2020). Today they are estimated to cover 15-17% of  
 137 vegetated land globally (Lehmann et al., 2019).



139 **Figure 1: Assemblies of Andropogoneae from throughout the phylogenetic and geographic range have**  
140 **varying divergence, ploidy, and genome size. A)** Species phylogeny built from 7,725 syntenic genes,  
141 including multiple copies in polyploids, using ASTRAL-PRO3. Pie charts at nodes show the quartet  
142 support for the main topology in blue, indicated by the tree, the first alternative topology in teal, and  
143 the second alternative in yellow. Polyploidy events are shown in diamonds, with colors corresponding to  
144 ploidy. Throughout all figures, diploids are shown in yellow, tetraploids in purple, paleotetraploids in  
145 blue, and hexaploids in red. The x-axis position of diamonds reflect timing of divergence of parental  
146 genomes, so may predate estimated species divergence. The WGD shared by all Tripsacinae is shown  
147 with one point at the median parental divergence of all taxa except obligate annual subspecies in *Zea*  
148 *mays*. Subtribes are shown as gray boxes, with names listed when we sampled more than one  
149 representative. Single representative subtribes are: 1. Germainiinae, 2. Sorghinae, 3. Ischaeminae, 4.  
150 Apludinae, 5&6 are Incertae sedis pending taxonomic revision, and 7. Chrysopogoninae **B)** Haploid size  
151 in gigabases of assembly (circle) and TEs and tandem repeats (TR) (triangle), colored by ploidy as in **A**,  
152 with diploids in yellow. Scaffolded assembly size, including N's, shown with a gray circle. Individuals with  
153 \* after sample name represent haploid assemblies. Across all assemblies, average assembly size is 1.9  
154 Gb, and average repeat size is 1.5 Gb. **C)** Map showing collection locations for our 33 samples in points  
155 colored by ploidy, as in **A**. The range of all Andropogoneae species is shown in green, constructed from  
156 wild occurrences in AuBuchon-Elder et al. (2023). Digitized collections are limited in the Indian  
157 subcontinent, although Andropogoneae are abundant there (Welker et al., 2020). **D)** Heterozygosity  
158 between alleles versus synonymous substitutions between homeologs for each assembly, with circles  
159 designating allopolyploids, triangles autopolyploids, and squares diploids. Diploids, which do not have  
160 homeologs, are assigned a Ks value of 0. The gray line indicates a 1:1 relationship between  
161 heterozygosity and synonymous substitution rate. *Chrysoposon serrulatus* is excluded from this plot, as  
162 it showed elevated nucleotide substitutions arising from nanopore sequencing. As it can be difficult to  
163 associate an individual plant with a point, figures with each assembly highlighted are available at  
164 [https://mcstitzer.github.io/panand\\_assemblies/](https://mcstitzer.github.io/panand_assemblies/).

## 165 **Diversity within species reflects differences in reproductive mode and population histories**

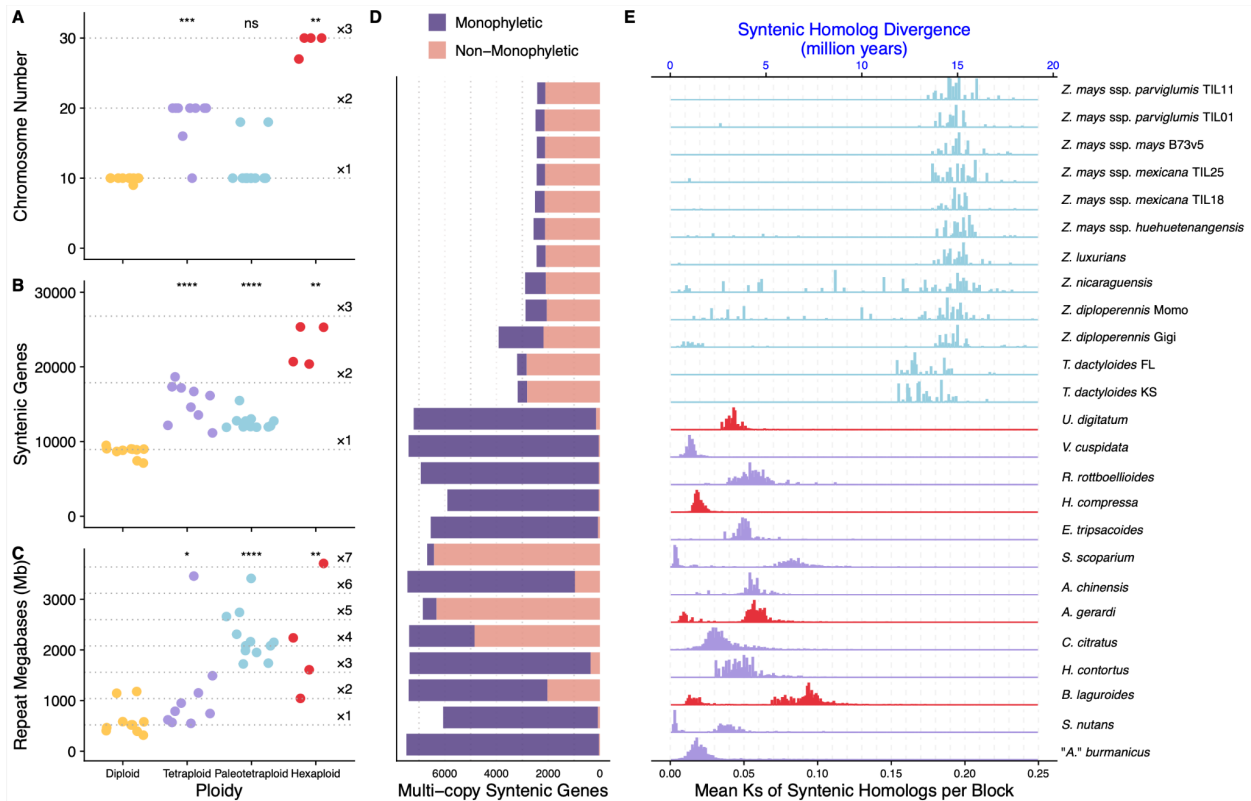
166 Comparison of alleles within individuals showed segregating variation ( $\pi$ ) across several  
167 orders of magnitude (from 0.0002% to 6%; Figure 1D), ranging from nearly homozygous  
168 individuals arising from self-fertilization, to high diversity consistent with large effective and  
169 census population sizes of these keystone grassland species. For example, low-heterozygosity  
170 *Anatherum virginicum* self-seeds extensively, facilitated by cleistogamous flowers (Campbell,  
171 1982), while high-heterozygosity *Heteropogon contortus* is a weedy apomict, found throughout  
172 tropical and subtropical latitudes (Carino & Daehler, 1999; Emery & Brown, 1958), and high-  
173 heterozygosity *Thelepogon elegans* has populations with permanent translocation  
174 heterozygosity facilitated by the formation of ring chromosomes (Sisodia, 1970). In general,

175 heterozygosity values are congruent with expectations from reproductive mode, life history,  
176 and range size (Supplemental Text).

### 177 **Polyploidy in Andropogoneae is common**

178 We characterized ploidy in our sequenced accessions by counting chromosomes and  
179 estimating copy number of syntenic regions derived from whole genome duplications (WGD).  
180 Amplification of genomes through polyploidy generates duplicated gene copies, inherited in  
181 blocks of conserved syntenic order along chromosomes. The number of times each syntenic  
182 region is present in a polyploid can thus give estimates of past WGDs. We used genes from  
183 diploid *Paspalum vaginatum* (Sun et al., 2022), which only shares ancestral grass WGDs with  
184 Andropogoneae, and is closely related in the sister tribe Paspaleae (Grass Phylogeny Working  
185 Group III, 2024). *Paspalum* genes are found in syntenic blocks with 1 to 6 copies in  
186 Andropogoneae, after adjusting values based on whether the assembly was haploid or allelic  
187 (as described in Li & Durbin, 2024). We integrated our results with cytological literature and  
188 chromosome counts to assign ploidy to our samples (Supplemental Figure S2; Supplemental  
189 Text). Our studied assemblies include 10 diploids, 9 tetraploids, 4 hexaploids, and 12  
190 paleotetraploids (cytological diploids arising from the Tripsacinae WGD; Figure 1). Their  
191 phylogenetic distribution provides fourteen independent polyploid formation events with  
192 which to study the impact of polyploidy on genome evolution.

193 Commonly observed consequences of polyploidy often include reductions in  
194 chromosome number, fractionation of duplicate genes, and expansions of transposable  
195 elements (Soltis et al., 2016; Wendel, 2015). In our analyses, we see elevated chromosome  
196 number in tetraploids and hexaploids (Figure 2A), variable reductions in duplicated genes in all  
197 polyploids (Figure 2B), and few expansions of repeat content beyond the multiplication  
198 expected from polyploidy (Figure 2C). Given that these patterns deviate from our expectations,  
199 we aimed to understand the genomic and temporal factors associated with polyploidy, to  
200 understand how these factors influence patterns of genome evolution.



201

202 **Figure 2: Polyploids in Andropogoneae are abundant.** **A)** Haploid chromosome number of each  
 203 sampled individual, versus ploidy. Diploid median and multipliers to tetraploid and hexaploid  
 204 expectations are shown with dotted lines. For **A-C**, statistical comparisons of each polyploid group to the  
 205 diploids were performed using a Wilcoxon rank-sum test. P-values for each comparison shown at top of  
 206 polyploid group, with \*\*\* p<0.001, \*\* p<0.01, \* p<0.05, and ns for nonsignificant. **B)** Number of syntenic  
 207 genes found in each individual, versus ploidy. Diploid median and multiplier to tetraploid and hexaploid  
 208 expectations are shown with dotted lines. **C)** Megabases of repeats in each sampled individual, versus  
 209 ploidy. Diploid median and multipliers up to 7x the diploid median are shown with dotted lines. **D)**  
 210 Relatedness of duplicate copies in each polyploid across 7,725 gene trees. Each bar matches labels in **E**.  
 211 Purple are gene trees where all tips of the given species are monophyletic, and pink are gene trees  
 212 where the tips are found in paraphyletic or polyphyletic (non-monophyletic) arrangements. As the  
 213 Tripsacinae paleotetraploidy is shared by multiple species, we downsampled gene trees so only the focal  
 214 Tripsacinae sample was present in the tree. **E)** Median synonymous substitutions (Ks) between syntenic  
 215 homologs in polyploids by alignment block, colored by ploidy.

216 Polyploids form with a mitotic or meiotic “catastrophe” (Comai, 2005), generating  
 217 gametes with additional sets of chromosomes. Similarity between the subgenomes derived  
 218 from these parental chromosomes varies from nearly identical (autopolyploid) to fully



219 distinguishable (allopolyploid) (Comai, 2005; Doyle & Egan, 2010; Kellogg, 2016; Ramsey &  
220 Schemske, 1998), but this relatedness exists along a continuum. When allelic diversity within  
221 subgenomes is indistinguishable from homeologous diversity between subgenomes, the species  
222 are likely autopolyploids ([Figure 1D](#); *Vossia cuspidata*, “*Andropogon*” *burmanicus*, *Hemarthria*  
223 *compressa*, *H. contortus*, *Cymbopogon citratus*; Individual species can be viewed at  
224 [https://mcstitzer.github.io/panand\\_assemblies/](https://mcstitzer.github.io/panand_assemblies/)). Each of these species is mixed-ploidy, and  
225 diploid cytotypes are known (Mehra 1982, Darlington and Janaki-Ammal, 1945). Further, *H.*  
226 *compressa* polyploids show formation of multivalents, supporting autopolyploid meiotic pairing  
227 (Gupta et al., 2017). Disomic inheritance in polyploids is facilitated by sequence divergence  
228 (Bingham, 1980; Mason & Wendel, 2020), as in allopolyploids where allelic diversity within  
229 subgenomes is lower than homeologous diversity between subgenomes (Figure 1D).  
230 Homeologous diversity in the remainder of Andropogoneae polyploids is consistent with  
231 allopolyploidy, with divergent gene copies captured from each parental species (Figure 1D).  
232 Allopolyploidy also leaves a signature in gene trees. If the parental taxa are extant and sampled,  
233 gene copies in the polyploid will be more closely related to their diploid progenitor than to the  
234 other polyploid (homeologous) copy; we call such gene tree patterns “non-monophyletic” with  
235 respect to the subgenomes. Our taxon sample places a majority of non-monophyletic  
236 relationships of gene copies in four polyploidy events (Figure 2D; *Tripsacinae*, *Andropogon*  
237 *gerardi*, *Schizachyrium scoparium*, *C. citratus*), strongly supporting allopolyploidy in each case.  
238 Two more polyploids have appreciable proportions of non-monophyletic gene trees (Figure 2D);  
239 *Andropogon chinensis*, *Bothriochloa laguroides*), likely reflecting our sampling of more distant  
240 relatives of potential allopolyploid parents. The distinction between auto and allopolyploidy can  
241 be hazy, as evidenced by *C. citratus* with low allelic diversity but with a closely related sampled  
242 congener *Cymbopogon refractus*, making a single label difficult.

243 To estimate the time the parents of these allopolyploids diverged, we used synonymous  
244 site divergence ( $K_s$ ) between homeologous copies (Figure 2E). Using a grass mutation rate  
245 ( $6.5e-9$ , Gaut et al., 1996) and assuming clock-like substitution rates, median divergences range  
246 from 1.05 Mya in *V. cuspidata* (median  $ks=0.014$ ) to the *Tripsacinae* WGD at 12.33 Mya (median  
247 perennial  $ks=0.160$ ). Although the *Tripsacinae* WGD is shared between *Zea* and *Tripsacum*

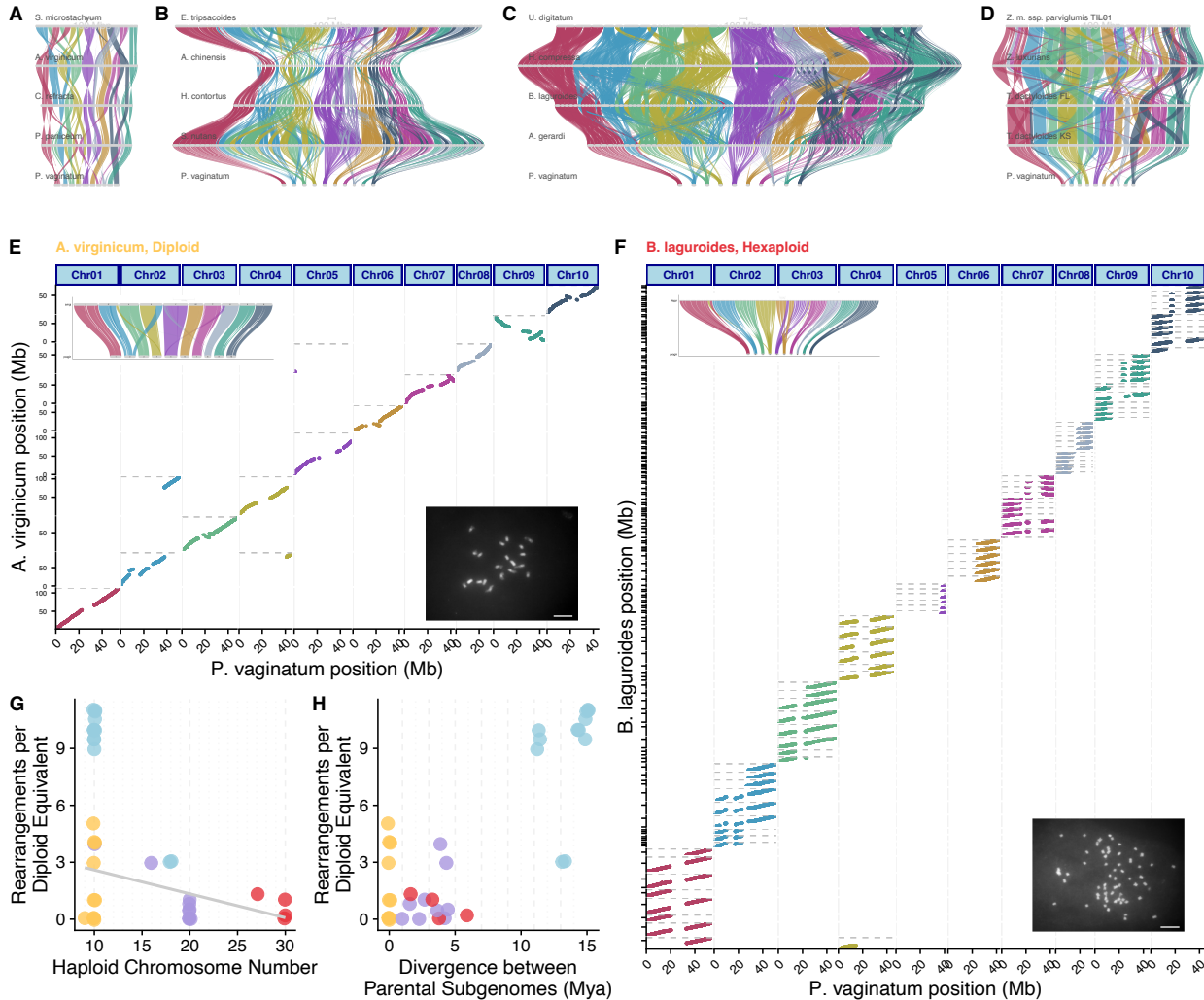
248 (Estep et al., 2014; McKain et al., 2018), subspecies within *Zea mays* have a derived annual life  
249 history (Doebley, 1990; Kempton & Popenoe, 1937), which is expected to increase the number  
250 of generations per year and hence estimated divergence (Figure 2E).

251 The divergence of parental genomes sets an upper limit on the timing of polyploid  
252 formation, but does not necessarily reflect when the polyploid nucleus was established  
253 (Kellogg, 2016). For instance, while the subgenome progenitors of tetraploid wheat diverged  
254 seven million years ago, they formed a tetraploid nucleus only ~800,000 years ago (Marcussen  
255 et al., 2014). Using SubPhaser (Jia et al., 2022), we identified divergent repetitive sequences  
256 between homeologous sequences, focusing on subgenome-specific TEs. However, only one  
257 assembly, the hexaploid *Urelytrum digitatum*, assigned homeologous sequences into the  
258 expected number of groups based on ploidy and chromosome count. In this species, diploid  
259 progenitors diverged 1.3 million years before polyploid formation (Supplemental Figure S3).  
260 The lack of differentiation in the remaining allopolyploids likely reflects uniform TE invasion and  
261 turnover following polyploidization, with insertions distributed evenly across chromosomes.

## 262 **Chromosome stability is higher in polyploids than diploids**

263 Chromosome number is a powerful yet simple descriptor of recent polyploidy. Elevated  
264 homologous chromosome number poses challenges to cellular processes like mitosis and  
265 meiosis (Comai, 2005), so is often countered by chromosome fusions and other rearrangements  
266 that reduce chromosome number (Mandáková & Lysak, 2018; Tayalé & Parisod, 2013). Rapid  
267 reduction of chromosomes in a newly formed allopolyploid can occur in tens of generations  
268 (Buggs et al., 2012; Xiong et al., 2011), and rediploidization via reduced chromosome number  
269 appears to be a general consequence of polyploidy, evidenced by its prevalence across  
270 flowering plants (Bowers & Paterson, 2021; J. F. Wendel, 2015). Most Andropogoneae  
271 genomes, including diploids (Figure 3A), tetraploids (Figure 3B), and hexaploids (Figure 3C) are  
272 almost entirely collinear to *Paspalum*, with limited rearrangements. However, paleotetraploid  
273 *Tripsacum dactyloides* show more rearrangements (Figure 3D), and all *Zea* individuals (Figure  
274 3D) share a massively rearranged karyotype with a reduction to 10 haploid chromosomes. In

275 contrast to an expectation of rediploidization via chromosome reduction, most Andropogoneae  
 276 polyploid species show remarkable chromosome stability.



277  
 278 **Figure 3: Chromosome stability is higher in polyploids than diploids in Andropogoneae.** A-D Genespace  
 279 riparian plots showing synteny of four genomes of each ploidy level, with *Paspalum* on the bottom.  
 280 Syntenic blocks of each *Paspalum* chromosome are shown as ribbons for **A)** Diploids, **B)** Tetraploids, **C)**  
 281 Hexaploids, and **D)** Paleotetraploids. **E)** Dotplot of syntenic anchor genes in blocks of 20 or more genes  
 282 from *Paspalum* chromosomes versus diploid *A. virginicum* chromosomes. The *A. virginicum* assembly is  
 283 haploid, so only homologous regions are present for each *P. vaginatum* chromosome. Inset in the top  
 284 left shows a riparian plot comparing chromosomes, and inset in bottom right shows the karyotype of *A.*  
 285 *virginicum* with  $2n=2x=20$ , scale bar 10  $\mu\text{m}$ . **F)** Dotplot of syntenic anchor genes in blocks of 20 or more  
 286 genes from *Paspalum* chromosomes versus hexaploid *B. laguroides* scaffolds. The *B. laguroides*  
 287 assembly has all six alleles assembled, so each homologous region can be present six times for each *P.*  
 288 *vaginatum* chromosome. Inset in top left shows riparian plot comparing assemblies, and inset in bottom  
 289 right shows karyotype of *B. laguroides* with  $2n=6x=60$ , scale bar 10  $\mu\text{m}$ . **G)** Chromosomal

290 rearrangements show a negative relationship to haploid chromosome number. Each assembly is  
291 represented by a point, diploids in yellow, tetraploids in purple, paleotetraploids in blue, and hexaploids  
292 in red, as in Figure 1. **H)** Rearrangements are not significantly related to time since divergence of  
293 polyploid parents. For **G** and **H**, the median value within each species *Zea* and *Tripsacum* was used for  
294 calculating the relationship, due to multiple sampling of this polyploidy.

295 Contemporary research suggests  $x=10$  is the base chromosome number of  
296 Andropogoneae (Spangler et al., 1999), and the majority of polyploids retain multiples of this –  
297 only five polyploidy events show chromosome fusions that give rise to reduced chromosome  
298 counts (Supplemental Table S3). Among these, only in *Zea* species and *Elionurus tripsacoides*  
299 does the reduction reinstate the base diploid chromosome number. To quantify  
300 rearrangements, we counted synteny breakpoints that merged two *Paspalum* chromosomes in  
301 a single scaffold of each assembly. Diploids have an average of 2 rearrangements, with no  
302 significant differences from the mean of tetraploids (1.67 rearrangements), and hexaploids  
303 (1.88 rearrangements), while paleotetraploids in *Zea* (mean 20.3 rearrangements) and  
304 *Tripsacum* (mean 6 rearrangements) have significantly higher values. However, on a per-  
305 chromosome basis, polyploids have fewer rearrangements. When scaled to match a diploid  
306 chromosome complement, chromosome number is negatively associated with rearrangement  
307 count ( $R^2=-0.416$ ;  $p=0.035$ ; Figure 3G), suggesting that rather than promoting chromosomal  
308 instability, polyploidy stabilizes the chromosome complement. Further, rearrangement  
309 abundance does not appear to be solely due to differential amounts of time for rearrangements  
310 to occur, as the two do not have a significant relationship ( $p=0.28$ ; Figure 3H). The underlying  
311 mechanisms of this observed retention of elevated chromosome number among most  
312 Andropogoneae polyploidy events remain unclear. It may involve selection for gene flow from  
313 mixed ploidy populations within the species (Kolář et al., 2017), or a filtering effect that favors  
314 the survival of only meiotically and mitotically stable polyploids (Otto, 2007; Ramsey &  
315 Schemske, 2002).

316 The ten *Zea* chromosomes show extensive rearrangements, including several  
317 integrations of an entire ancestral chromosome to the center of another (Figure 3D;  
318 Supplemental Figure S4), as seen previously (H. Wang & Bennetzen, 2012). Yet aside from  
319 inversions and rare translocations, the *Zea* karyotype is unchanged throughout the genus (Braz

320 et al., 2020; Laurie & Bennett, 1985). Of the 14 rearrangements that differentiate *Zea* from  
321 *Tripsacum*, seven contain tandemly repeated knob sequences within one megabase of the  
322 breakpoint, a major enrichment relative to their average of 1.1% of chromosomal sequence. In  
323 maize, chromosomal knobs can act as neocentromeres, mediating meiotic drive by favoring  
324 their own segregation (Buckler IV et al., 1999; Dawe et al., 2018; Rhoades, 1942). Such tandem  
325 repeats have been linked to genomic shock, as in response to broken chromosomes or cell  
326 culture (Lee & Phillips, 1987; McClintock, 1941, 1984; Rhoades & Dempsey, 1972). The tandem  
327 array of genes responsible for meiotic drive arose contemporaneously with the divergence of  
328 the two genera (L. Chen et al., 2022; Dawe et al., 2018), suggesting meiotic drive may have  
329 initiated *Zea*'s pronounced chromosomal rearrangements. However, across Andropogoneae, TE  
330 and tandem repeat content is not significantly correlated to rearrangement count  
331 (Supplemental Figure S5). These findings suggest that while large tandem arrays are prone to  
332 rearrangements, their presence alone does not drive them, as many taxa with large arrays show  
333 no rearrangements (Figure 5D). Interestingly, cytological constrictions at knobs have been  
334 observed in the genus *Elionurus* (Celarier, 1957; Supplemental Text), the only other instance  
335 where we observe a major reduction in chromosome number.

### 336 **Most genes are retained in multiple copies, but regulatory sequence turns over rapidly**

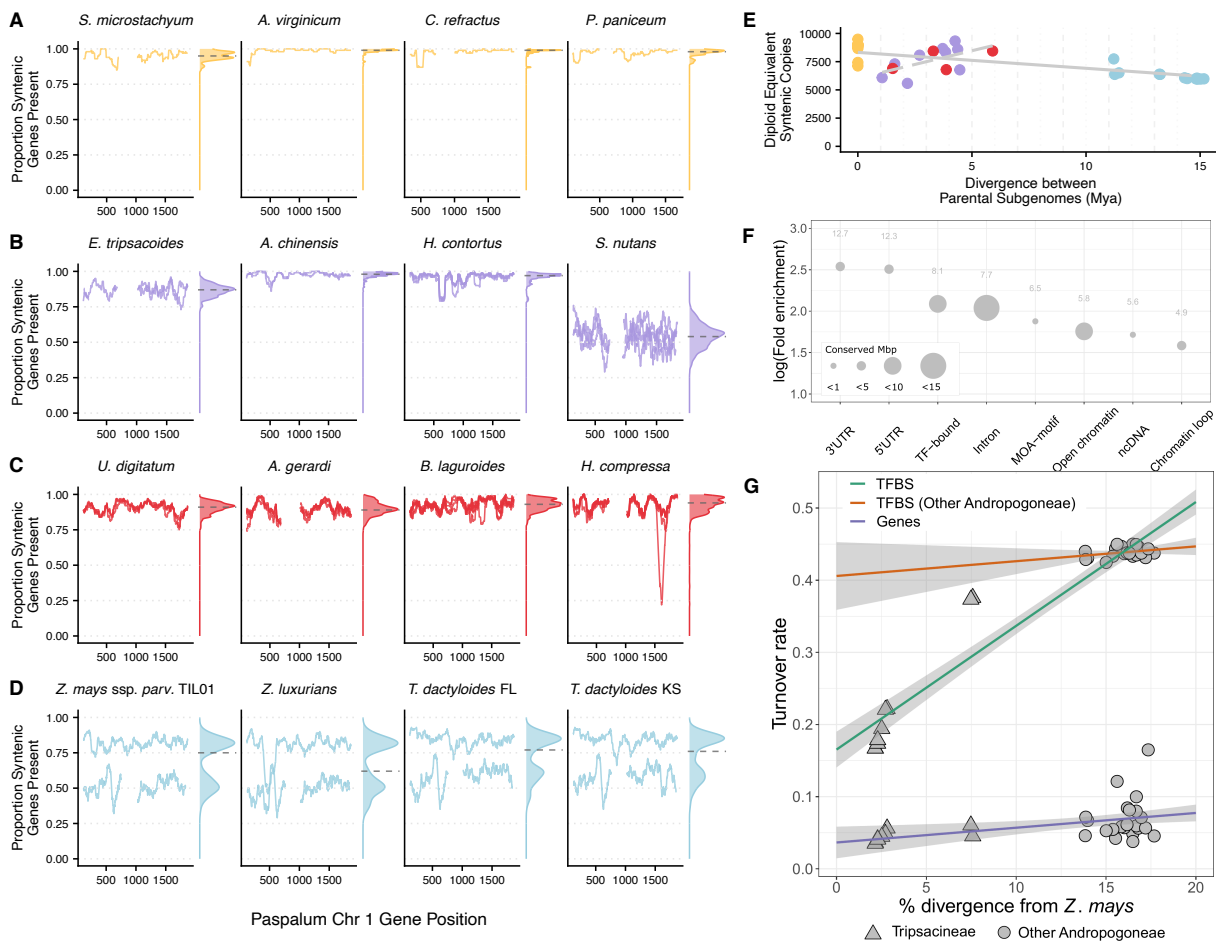
337 After a whole genome duplication, functional redundancy leads to decay of  
338 homeologous copies, but stoichiometry of protein complexes and pathways leads to  
339 preferential retention of some duplicates, particularly transcription factors, developmental  
340 genes, and proteins in multi-protein complexes (Birchler & Veitia, 2012; Blanc & Wolfe, 2004;  
341 Freeling, 2009). To investigate duplicate retention in our species, we standardized copy number  
342 to the genome median, then identified genes showing differential copy number between  
343 diploids and polyploids, and conducted a gene ontology (GO) analysis on the top 100 genes. The  
344 most strongly enriched GO category included genes encoding ribosomal proteins (“polysomal  
345 ribosome,” GO:0042788), consistent with constrained stoichiometry of interacting subunits  
346 (Birchler & Veitia, 2012) and with observations of retained duplicates in other plant polyploids  
347 (Barakat et al., 2001; Rosado & Raikhel, 2010; Roulin et al., 2013). Signal transduction and

348 stress-response categories were also enriched (Supplementary Table S4). Several  
349 developmental processes showed elevated copy number in polyploids, notably root hair  
350 initiation, including a gene homologous to brittle culm 10 of rice (Zhou et al., 2009). Increased  
351 root hair density following polyploidization has been observed in both *Arabidopsis* (Stetter et  
352 al., 2015) and wheat (Han et al., 2016), particularly under nutrient-poor conditions (Salazar-  
353 Henao et al., 2016). Other enriched developmental genes relate to cellulose deposition,  
354 potentially reflecting the increased mechanical demands on cell walls that accompany altered  
355 cell volumes (Corneillie et al., 2019; Morrison, 1980; Serapiglia et al., 2015).

356 Immediately after polyploidy, all genes exist in multiple copies. Subsequent loss of  
357 duplicates can occur through genetic drift (Lynch & Conery, 2000), or may arise from selective  
358 processes, such as targeted gene removal (Paterson et al., 2006) or the resolution of dosage  
359 conflicts (De Smet et al., 2013; Edger & Pires, 2009). In Andropogoneae, polyploid gene counts  
360 suggest such loss, with fewer syntenic genes than suggested from a multiplication of the diploid  
361 genome (Figure 2B, 2D, Supplemental Figure S6). To determine whether gene loss is a gradual  
362 neutral process, we relate this diploid-equivalent gene count to time since polyploidy, as  
363 measured by parental divergence. We observed a negative correlation ( $p=0.008$ ,  $R^2=0.23$ ,  
364 Figure 4E), with a slope implying a loss of 141 genes per million years. However, among  
365 tetraploids and hexaploids there is a positive correlation ( $p=0.03$ ,  $R^2=0.301$ ), such that older  
366 polyploids retain more duplicates. This suggests a filtering effect – polyploid lineages that  
367 persist over time may do so by maintaining more of their duplicate genes.

368 We next asked whether subgenomic ancestry impacts the gene loss that does occur.  
369 When more genes are lost from one homeologous chromosome, it is often attributed to biased  
370 fractionation. The maize genome is a classic example of this process, with almost two times as  
371 many duplicate genes retained in one subgenome as the other (Schnable et al., 2011). To  
372 explore biased fractionation among our polyploids, we measured gene retention in 100 gene  
373 windows along *Paspalum* chromosomes for each assembly. Diploids show high retention of  
374 syntenic genes (Figure 4A), but most tetraploids (Figure 4B) and hexaploids (Figure 4C) also  
375 show near-complete retention of all duplicate copies. In contrast, all Tripsacinae  
376 paleotetraploids (Figure 4D) show uneven gene retention across subgenomes: *Tripsacum* retains

377 85% and 56.8% of genes on each subgenome, while *Zea* retains 81.5% and 49.5%, indicating  
 378 greater gene loss in *Zea*. Although some polyploids exhibit considerable gene loss (e.g., *S.*  
 379 *nutans* retains only 51.4%; Figure 4B), none show the degree of biased fractionation as seen in  
 380 Tripsacinae. Although lack of biased fractionation may be expected for autopolyploids (Zhao et  
 381 al., 2017) if recombination equalizes loss across chromosomes, we also found minimal  
 382 differential fractionation in many allopolyploids. Genome-wide retention of gene copies  
 383 suggests that polyploidy may act to retain dosage of quantitative traits.  
 384



385  
 386 **Figure 4: Genes are stagnant while noncoding regulatory sequence turns over rapidly. A-D)** Retention  
 387 of syntenic genes in 100 gene windows along *Paspalum* chromosome 1 genes in **A)** diploids with one  
 388 subgenome, **B)** tetraploids with two subgenomes, **C)** hexaploids with three subgenomes, and **D)**  
 389 paleotetraploids with two subgenomes. Density plot to the right of each genome shows the genome-  
 390 wide distribution of these values. **E)** Relationship between gene retention and time since polyploidy,  
 391 with overall loss (solid line), but a positive relationship within tetraploids and hexaploids (dashed line).  
 392 Regression for the solid line uses the median value across more densely sampled genera *Zea* and

393 *Tripsacum*. **F)** Enrichment of *Z. mays* genomic features relative to genomic abundance in our non-coding  
394 sequence most conserved across Andropogoneae. Absolute fold enrichment is displayed above each  
395 point. **G)** Turnover of predicted transcription factor binding sites (TFBS) and genes between *Z. mays* and  
396 other Andropogoneae species by genomic divergence. A single representative subgenome was used for  
397 TFBS turnover calculations in polyploid species. Loess smooth linear regression lines with 95%  
398 confidence intervals are shown. Genomic divergence was calculated using alignments to all fourfold  
399 degenerate sites in *Z. mays*.

400

#### 401 *Conservation and acceleration of genome-wide conserved elements*

402 Polyplodization duplicates not only genes, but also noncoding regions, expanding the  
403 potential for regulatory diversification and expansion (Ebadi et al., 2023; Osborn et al., 2003).  
404 To assess the conservation of noncoding sequences, we generated a syntenic multiple  
405 sequence alignment of all taxa. We identified 2,302,710 highly conserved Andropogoneae  
406 sequence elements covering 72.4 Mbp, enriched in genic regions and potential regulatory  
407 sequences associated with binding sites, accessible chromatin, and chromatin loops (Figure 4F,  
408 Supplemental Figure S7). After excluding genic sequences (coding sequence, introns, and  
409 untranslated regions (UTRs)), we found a set of 1,664,343 conserved non-coding sequences  
410 (CNS), each averaging 22 bp, and comprising a total of 36.2 Mbp (Supplemental Table S5),  
411 numbers consistent with previous characterizations of CNS in grasses (Liang et al., 2018; Song  
412 et al., 2021) and with functional evidence of conserved chromatin accessibility between maize  
413 and sorghum (Lu et al., 2019). The Tripsacinae showed substantial sequence-level divergence  
414 from other Andropogoneae, with only 34.8% of the non-repetitive maize genome aligning in at  
415 least half of the Andropogoneae species sampled, reflecting their deep divergence.  
416 Nevertheless, Andropogoneae CNS, including Tripsacinae-accelerated CNS, share features with  
417 CNS in other plants including their short size and association with developmental and  
418 transcriptional regulation genes (Burgess & Freeling, 2014).

419 Transcription factor binding sites (TFBS) are an important subset of noncoding  
420 sequences and were recently shown to explain the majority of phenotypic variation in many  
421 maize traits (Engelhorn et al., 2024). Despite their importance, TFBS turn over more rapidly  
422 than genes. For example, in Brassicaceae, CNS turnover is rapid (Haudry et al., 2013) and 74% of  
423 experimentally validated TFBS have turned over between *Arabidopsis thaliana* and *A. lyrata*



424 which diverged 10 Mya (Muiño et al., 2016). We investigated whether the Tripsacinae lineage  
425 has experienced accelerated evolution in CNS, finding 15,989 CNS with significant signal of  
426 acceleration (Supplemental Table S5). GO enrichment analysis revealed that CNS-associated  
427 genes were strongly enriched for core physiological and developmental processes  
428 (Supplemental Table S6). The Tripsacinae-accelerated CNS were also enriched for terms linked  
429 to development including “response to red or far red light” (GO:0009639, fold enrichment=2.7,  
430 adjusted p=0.001) and “photoperiodism, flowering” (GO:0048573, fold enrichment=2.1,  
431 adjusted p=0.006) (Supplemental Table S7). TFBS found in CNS were most strongly enriched for  
432 motifs of the auxin response factor ARF27 (motif MA1691.1, fold enrichment=1.5, adjusted  
433 p<1E-300), which is involved in developmental processes (Supplemental Table S8).

434 To further assess cis-regulatory region turnover across the Andropogoneae, we assessed  
435 pairwise TFBS turnover between *Z. mays* and the other Andropogoneae in 11,173 genes  
436 meeting our functional and conservation criteria (Methods). TFBS turnover was linked to overall  
437 sequence divergence (Figure 4G). Compared to *Z. mays*, the mean pairwise turnover of  
438 predicted TFBS is 0.19 in other *Zea* species, 0.37 in *Tripsacum* and 0.44 in other  
439 Andropogoneae. In contrast, the coding sequence of these same genes turned over 7 times  
440 slower, at a rate of 0.06 across all Andropogoneae. This suggests that the Andropogoneae  
441 experience rapid evolutionary turnover of TFBS. Because we focused only on putative  
442 regulatory regions that could be aligned between species and thus exclude highly diverged  
443 regions, the turnover rates we determined could be underestimates. However, the  
444 unexpectedly high rate of turnover of 19% between maize and the closely related teosinte  
445 species suggests that a substantial proportion of the predicted TFBS may not be functional,  
446 underlining the need for experimentally validated TFBS in maize to improve estimates of TFBS  
447 turnover.

448

#### 449 *Genes are further apart in polyploids*

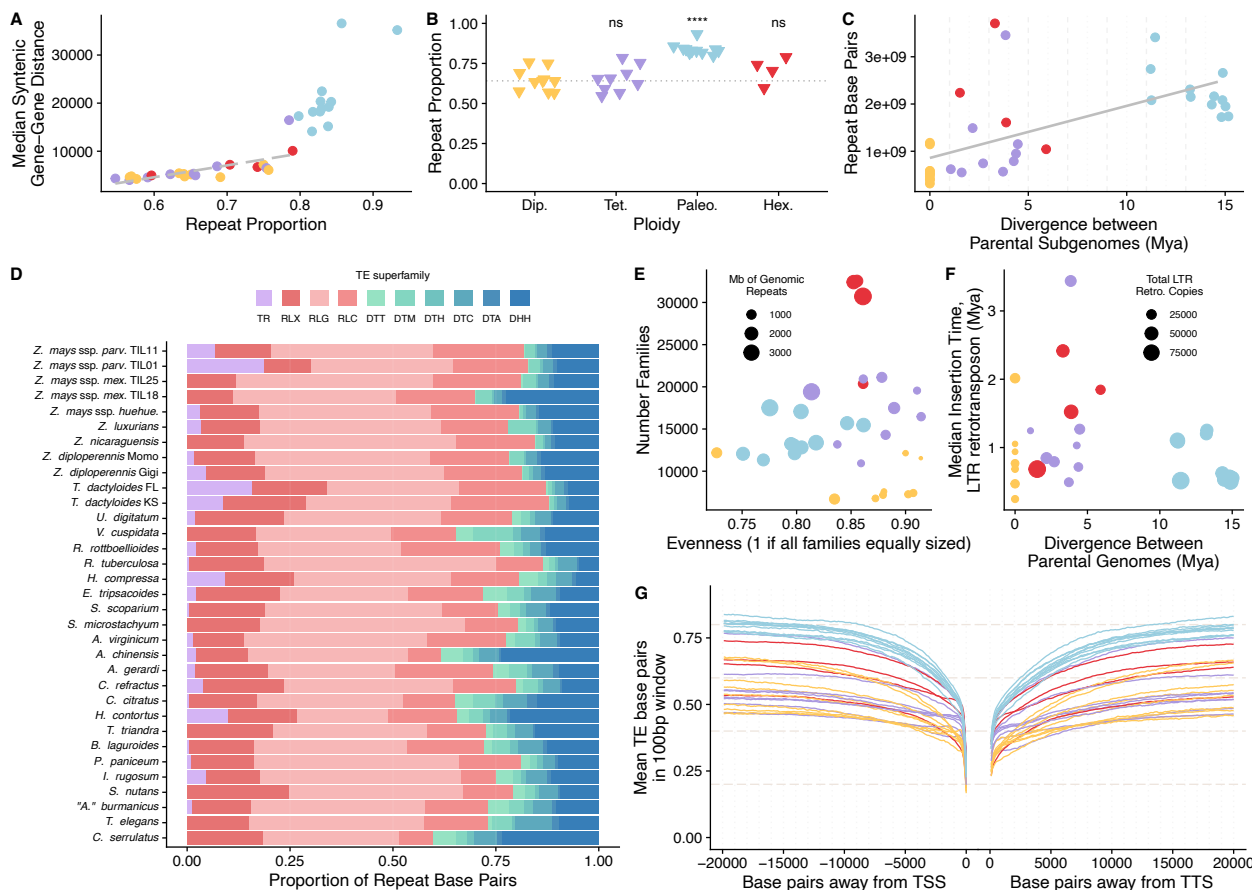
450 This rapid turnover of TFBS over relatively short timescales led us to explore potential  
451 drivers, with TEs emerging as key candidates. The maize genome has been described as having  
452 tight clusters of genes separated by large swaths of retrotransposons (Fu & Dooner, 2002;

453 Morgante et al., 2005; SanMiguel & Bennetzen, 1998), and across plants, high-density gene  
454 regions seem to be evenly spaced independent of genome size (Feuillet & Keller, 1999; Llaca &  
455 Messing, 1998). This arrangement suggests that critical regulatory sequences are compressed  
456 within short intergenic regions and maintained by selection. We measured median gene-gene  
457 distance of syntenic genes across ploidy levels, finding similar values in diploids (4.98 kb) and  
458 tetraploids (5.21 kb apart), slightly larger in hexaploids (6.95 kb), but greater in paleotetraploids  
459 (19.06 kb) (Supplemental Figure S8). This tighter spacing in diploids and tetraploids aligns with  
460 *A. thaliana*'s ~5kb gene density (Kellogg & Bennetzen, 2004). Gene spacing shows a strong  
461 correlation with repeat content (Figure 5A) when excluding paleotetraploids, with genome size  
462 alone explaining 72% of the variance in median gene-gene distances ( $p=2.04e-07$ ). These  
463 findings suggest that TE activity may be expanding intergenic regions in paleotetraploid  
464 Tripsacinae, introducing new TFBS and disrupting existing ones.

#### 465 **Polyploidy is not associated with TE bursts**

466 Besides polyploidization (Figure 2C), the primary driver of increases in genome size  
467 between plant taxa is the amplification of transposable elements (Bennetzen et al., 2005;  
468 Bennetzen & Kellogg, 1997; Pulido & Casacuberta, 2023). The genomic shock of allopolyploidy  
469 has been hypothesized as a force that can release silencing of TEs, allowing them to expand  
470 (McClintock, 1984; Pikaard, 2001). Our data show diploids have lower proportions of their  
471 genome coming from TEs (mean=64.8%) than polyploids (mean=75.0%) ( $p=0.0043$ ) (Figure 5B),  
472 but this difference is driven by the paleotetraploid Tripsacinae species and is no longer  
473 significantly different after removing them (tetraploid and hexaploid mean=66.9%,  $p=0.509$ ).  
474 This suggests no global expansion of TEs following polyploidy, consistent with certain  
475 reconstituted interspecific and intergeneric hybrids (Parisod et al., 2010), young polyploidy  
476 events like wheat (Papon et al., 2023), and old polyploidies in animal genomes (Mallik et al.,  
477 2023). Despite this general pattern, specific Andropogoneae lineages do exhibit massive TE  
478 expansions, most notably *U. digitatum*, which has the largest genome among our samples.  
479 Rather than the three-fold expansion expected from hexaploidy, it has over seven times more  
480 TE base pairs than expected given the diploid median (Fig 2C). *E. tripsacoides* and *H. compressa*

481 also show large increases in repeat bases relative to diploid multiplications. Thus, while  
 482 polyploidy may enable TE expansion in some lineages, it does not guarantee it. We modeled TE  
 483 accumulation as a function of time since polyploidy, and observed a positive correlation, with  
 484 an estimated gain of 110 Mb of TE sequence per million years ( $R^2=0.23$ ,  $p=0.008$ , Figure 5C).  
 485 This rate exceeds the 38 Mb per million years estimated for diploid rice (Bennetzen et al., 2005;  
 486 Ma & Bennetzen, 2004), but additional comparative studies across taxa are warranted. Overall,  
 487 these data do not support global unregulated transposition upon polyploid formation, rather, a  
 488 gradual accretion of TEs as the polyploid establishes through evolutionary time.



489  
 490 **Figure 5: Transposable elements react stochastically to polyploidy.** **A)** Proportion of the genome in  
 491 repeat sequence versus the median distance between syntenic genes. Points are colored by ploidy, with  
 492 diploids in yellow, tetraploids in purple, paleotetraploids in blue, and hexaploids in red. The dashed line  
 493 shows regression excluding paleotetraploids, with a strong positive correlation ( $r=0.71$ ,  $p=0.00016$ ). **B)**  
 494 Repeat proportion related to ploidy level. Statistical comparisons of each polyploid group to the diploids  
 495 were performed using a Wilcoxon rank-sum test. P-values for each comparison are shown at the top of  
 496 each polyploid group, with \*\*\*\*  $p < 0.0001$  and ns for nonsignificant. Diploid median is shown as a dotted  
 497 horizontal line. **C)** Divergence between parental subgenomes versus repeat base pairs in each assembly.

498 The solid line includes the median value of *Zea* and *Tripsacum*, which are positively correlated ( $r= 0.51$ ,  
499  $p= 0.008$ ). **D**) Proportion of repeats in each genome belonging to different TE superfamilies. TR is  
500 Tandem Repeats of all length classes, red colors show LTR retrotransposons (RLX-Unknown; RLC-  
501 Ty1/Copia; RLG-Ty3), and blue colors DNA transposons (DTT-Tc1/Mariner; DTM-Mutator; DTH-  
502 pIF/Harbinger; DTC-CACTA; DTA-hAT; DHH-Helitron). **E**) Pielou's evenness metric versus number of  
503 families in each assembly. These are calculated only including families with at least 10 copies in the  
504 genome. The evenness metric ranges from 0 (one family contributing all copies) to 1 (all families equally  
505 sized). Points are scaled by the amount of repeat base pairs in the genome, and colored by ploidy. **F**)  
506 Divergence between parental subgenomes and median timing of amplification of LTR retrotransposons.  
507 Point size is scaled by the number of structurally intact LTR retrotransposons identified in the genome,  
508 and colored by ploidy. **G**) Mean TE base pairs in 100 bp windows away from the transcriptional start site  
509 (TSS), left, and transcriptional termination site (TTS), right, of all Helixer genes, colored by ploidy.

510  
511 Polyploidy can disrupt the epigenetic environment of the cell (Doyle & Coate, 2019), and  
512 these alterations can allow lineages of TEs that were well-silenced in diploid progenitors to  
513 exploit novel vulnerabilities in epigenetic silencing. These amplifications are often observed  
514 when single TE families reach high copy number (Baduel et al., 2019; J. Chen et al., 2020;  
515 Hawkins et al., 2006; Tsukahara et al., 2009). We first identified variation in the proportion of  
516 the genome coming from each TE superfamily across Andropogoneae (Figure 5D). As observed  
517 in all grass genomes (Vicient et al., 2001), LTR retrotransposons, and particularly Ty3 LTR  
518 retrotransposons, contribute the highest proportion of repeat sequence. This simple  
519 description of superfamily abundance showed proportional expansions of gene-proximal DNA  
520 transposons in specific species, like DTM Mutator elements in *V. cuspidata* and DHH Helitron  
521 elements in *Chrysopogon serrulatus* and *A. chinensis*.

522 To understand whether TE amplifications involved many families or were driven by just  
523 a few, we applied species diversity metrics to explore the relative abundance of TE families in  
524 each genome. Pielou's evenness ranges from 0 (where a single family dominates) to 1 (where  
525 all families are equally represented). Although evenness varies among genomes (Figure 5E),  
526 only paleotetraploids show significantly different evenness compared to other ploidy groups  
527 (Wilcoxon rank sum test,  $W=240$ ,  $p=2.45e-05$ ). Species with high evenness tend to have lower  
528 repeat content (Figure 5E), supporting a lack of successful bursts that overcome epigenetic  
529 silencing.

530 We were curious whether the timing of TE amplification could be linked to polyploidy.  
531 However, the rapid turnover of TEs in genomes makes it challenging to directly identify those  
532 inserted during polyploidy (Fedoroff, 2012; Vitte & Bennetzen, 2006; Wicker et al., 2018), as  
533 repeat landscapes are constantly overwritten. To address this, we estimated the mean age of  
534 LTR retrotransposons in the genome by measuring the divergence between their LTRs  
535 (SanMiguel et al., 1998). Our analysis reveals a general trend – that diploids have younger LTR  
536 retrotransposons, while polyploids harbor older copies, with no clear relationship to the timing  
537 of polyploidy (Figure 5F). Young TE insertions have been shown to be more deleterious than  
538 older ones (Stitzer et al., 2023), contributing disproportionately to genetic load. As a result, the  
539 buffering effect of polyploidy masking deleterious TE insertions may provide an adaptive  
540 benefit in these grasses.

541 Much of the impact of TEs on genome function is via their relationship to generating  
542 genetic diversity near genes. We measured the proportion of sequence 20 kilobases upstream  
543 and downstream (Figure 5G) of genes that is occupied by TEs. Although all species plateau to  
544 values near genome-wide averages 20 kb from genes, the shape of the relationship near genes  
545 differs (Figure 5G). For example, diploids and tetraploids reach 50% TEs at a median of 9 kb and  
546 8.4 kb away from the 5' UTR of the gene, while paleotetraploids (0.5kb) and hexaploids (0.8kb)  
547 do so much closer. TEs are well known to affect gene expression (Hirsch & Springer, 2017; Lisch,  
548 2013), disrupting regulatory sequence and introducing new ones. We anticipate incorporating  
549 these differences in TE content in key regulatory regions near genes will help understand  
550 differences in gene expression.

## 551 552 **Conclusion**

553 Together, these observations underscore a general stasis of genome evolution in these  
554 33 newly assembled Andropogoneae grasses, even under the altered genomic environment of  
555 polyploidy. Contrary to the expected patterns of chromosome reduction, gene loss, and TE  
556 amplification, most polyploid events deviate from these “rules.” This long-term genomic  
557 stability may be facilitated by perennial life cycles of these grasses, which supports persistence

558 on the landscape and exploration of diverse allele combinations, enhancing their adaptive  
559 potential.

560 While polyploidy can sometimes reduce fitness, its broader benefits, beyond simple  
561 genome doubling, include fixed heterozygosity, buffering against deleterious genetic load, and  
562 increased phenotypic flexibility through gene dosage (Doyle & Coate, 2019; Stebbins, 1971;  
563 Tayalé & Parisod, 2013). The large census population sizes, wind-pollination, and high fecundity  
564 of *Andropogoneae* grasses likely enable the exploration of genotypic space, potentially purging  
565 genetic load thought to accumulate in polyploids (Haldane, 1933) and exploring gene dosage  
566 landscapes.

567 Modern maize cultivation epitomizes the benefits of hybridization, leveraging heterosis  
568 from the combining ability of divergent parental gene pools (Duvick, 2001). Studying how  
569 distant relatives of maize adapt to the permanent heterozygosity of polyploidy can provide  
570 valuable insights into the complexities of heterosis and its evolutionary significance.

571

## 572 **Methods**

### 573 ***Germplasm***

574 Plant material was sourced from seed banks or collected in the wild (Supplemental  
575 Table S1), and grown in the greenhouses at the Donald Danforth Plant Science Center in St.  
576 Louis, MO, Cornell University in Ithaca, NY, and the University of California, Davis, in Davis, CA.  
577 Permits for collection, export and import were obtained as specified by local governments and  
578 nature reserves. Most plants were grown to flowering and voucher specimens were collected  
579 and deposited at the herbarium of the Missouri Botanical Garden (MO) and the Australian  
580 National Herbarium (CANBR). All specimens were imaged, with the image and metadata  
581 uploaded to the Tropicos database following standard protocols (Supplemental Table S1).

582

### 583 ***DNA Extraction and Library Preparation***

584 Approximately 5 g of fresh tissue from each plant was extracted for PacBio (Pacific  
585 Biosciences, USA) sequencing using one of three high molecular weight DNA approaches. One  
586 was based on the Circulomics Big DNA Kit (Circulomics, USA), another on Doyle and Doyle  
587 (1987), and another based on the Macherey-Nagel NucleoBond kit (Macherey-Nagel, USA). This  
588 DNA was used to generate PacBio libraries, after size selection with either BluePippin (Sage  
589 Science, USA), PippinHT (Sage Science, USA), or Ampure beads (Beckman Coulter, USA).  
590 Sequencing was completed on the Sequel II or Sequel IIe across 1 to 8 flow cells depending on  
591 genome size and output of individual sequencing runs. DNA extraction and sequencing were  
592 completed by Corteva Agriscience, Arizona Genomics Institute, and the USDA Genomics and  
593 Bioinformatics Research Unit, Stoneville, MS. Nanopore reads for *C. serrulatus* were previously  
594 generated (Song et al., 2021).

595

### 596 **Optical Map and HiC Generation**

597 For optical map construction, DNA was extracted from ~ 0.7 g of fresh leaf tissue from  
598 each plant using agarose embedded nuclei and the Bionano Prep Plant Tissue DNA Isolation kit  
599 (Bionano, USA). DNA extraction, labeling, and imaging followed the methods previously  
600 described in Hufford et al., 2021 and was completed by Corteva Agriscience.

601 For Hi-C Sequencing, *Tripsacum dactyloides* FL chromatin was crosslinked and isolated  
602 from approximately 1g frozen leaf tissue. One Hi-C Seq library was constructed using the  
603 Proximo system (Phase Genomics, Seattle) according to the manufacturer's recommendations  
604 and sequenced in a PE150 format in an Illumina Novaseq 6000 analyzer.

605

### 606 **Genome Assembly**

607

608 We used three sequencing technologies, PacBio Hifi, PacBio CLR, and Oxford Nanopore  
609 MinION. Each was assembled into contigs using the detailed methods below (Supplemental  
610 Table S2). For 24 samples, we used additional information to further scaffold contigs. This took  
611 the form of Bionano optical maps, and HiC for one *Tripsacum dactyloides* individual. For taxa in  
612 the genus *Zea*, we used pan-genome anchors to further scaffold into chromosomes.

613

#### 614 *Contig assembly*

615 We generated PacBio Hifi data for 27 individuals, and generated contig assemblies using  
616 Hifiasm. HiFi reads obtained from the Circular Consensus Sequencing (CCS) (v6.4.0) pipeline  
617 were converted to FASTA format, and Hifiasm (v0.19.5-r590) (Cheng et al., 2021) was used to  
618 assemble contigs from the FASTA input reads, using parameters dependent on the scaffolding  
619 information available.

620 For genomes with only PacBio HiFi data, we set the purge level to 3 (-l 3) to generate  
621 contigs, purging haplotigs in the most aggressive way. The assembly graph of primary contigs  
622 (\*.bp.p\_ctg.gfa) was used as the representative set of contigs.

623 For genomes with PacBio HiFi data plus a BioNano optical map, we set the purge level to  
624 0 (-l 0) to prevent the purging of duplicate haplotigs. This generated primary and alternative  
625 haplotypes, which were combined to generate contigs.

626 For the *T. dactyloides* FL genome with HiC data, we used Hi-C partitioning, supplying the  
627 HiC FASTQ reads (-h1 -h2), with purge level set to 3 (-l 3). This generated two phased contig  
628 assemblies.

629 We generated PacBio Continuous Long Read (CLR) data for 5 individuals, and generated  
630 contig assemblies using Canu (v1.9) (Koren et al., 2017). Pacbio (CLR) data were converted from  
631 the native output (Binary Alignment Map, or BAM) format to FASTA format using  
632 samtools(v1.17) (Danecek et al., 2021) fasta subcommand, and were then error corrected using  
633 Falcon (v1.8.0) (Chin et al., 2016). Briefly, Falcon's first stage (overlap detection and error  
634 correction module) was run, specifying genome size (-genome\_size, for auto coverage  
635 estimation) and with error correction options of a minimum of two reads, maximum of 200  
636 reads, minimum identity of 70% for error corrections, average read correction rate set to 75%,  
637 and maximum seed coverage of 40X, and a chunk size for local alignments of at least 3,000 bp.  
638 For the DALigner step, the identical kmer match length was set to 18 bp (-k 18) with a read

639 correction rate of 80% (-e 0.80) and local alignments of at least 1,000 bp (-l 1000). Contigs were  
640 generated using Canu (v1.9), after merging the error-corrected reads from Falcon jobs, using  
641 the default options except for ovlMerThreshold=500 (kmers that occur more than 500 times are  
642 not used as seeds).

643 We used Oxford Nanopore MinION reads for one individual, *Chrysopogon serrulatus*  
644 (Song et al., 2021). Basecalling was performed using Guppy (v 2.1.3), and FASTQ files were used  
645 for error correction. The porechop package (Wick et al., 2017) was used to clean for adapter  
646 trimming and error correction of ~ 52 Gb of MinION reads. The reads were then assembled  
647 using Canu v1.8 with the default parameters as described above in the section on CLR, but with  
648 the default ovlMerThreshold.

### 649 650 *Scaffolding*

651 We generated Bionano optical maps for 21 individuals. The Bionano optical maps were  
652 processed using Bionano Solve (v3.4) and Bionano Access (v1.3.0), following the methodology  
653 outlined in Hufford et al., (2021). For the hybrid assembly, default settings from the  
654 configuration file (hybridScaffold\_DLE1\_config.xml) and parameters file (optAr-  
655 guments\_nonhaplotype\_noES\_noCut\_DLE1\_saphyr.xml) were utilized. The scaffolding phase in  
656 Bionano Solve incorporates 1) estimated gaps of varying N-size, excluding 100 bp or 13 bp gaps,  
657 as determined through calibrated distance conversion of the optical map to base pairs, 2)  
658 unknown gaps (100-N gaps), and 3) 13-N gaps, which are introduced when two contigs overlap.  
659 Due to polyploidy and high heterozygosity of many genomes, the 13-N gaps were curated  
660 manually, using Bionano Access (v1.3.0). Alignments of contigs to the optical map were  
661 examined in detail, and contigs were either trimmed near overlapping regions or exact  
662 duplicates were labeled as alternative haplotypes (e.g. alt-scaf\_NNN).

663 We generated Hi-C reads from *Tripsacum dactyloides* FL. Hi-C reads in FASTQ format  
664 were first mapped to both haplotypes of the phased haploid genomes of *Tripsacum dactyloides*  
665 FL (contigs assembled using Hi-C partitioning) using the Burrows-Wheeler Aligner (BWA)  
666 (v0.7.12) (H. Li & Durbin, 2009). The juicer pipeline (v1.6) (Durand et al., 2016) was used to filter  
667 out erroneous mappings (MAPQ = 0) and duplicates, and to generate the interaction matrix.  
668 The 3D-DNA pipeline (v180) (Dudchenko et al., 2018) was then used to anchor the contigs to  
669 chromosomes and error correct the contigs using default parameters. The resulting Hi-C  
670 contact maps were manually examined using JUICEBOX Assembly Tools (v2.15.07) (Dudchenko  
671 et al., 2018) and a few out-of-place contigs were manually corrected. A final assembly for each  
672 phased haplotype was generated and the genome with fewer Ns was designated as primary  
673 haplotype.

674 To scaffold the *Tripsacum dactyloides* KS genome, we used ALLMAPS (Tang et al., 2015)  
675 to order and orient the primary and alternative contigs. Briefly, haplotype 1 of *T. dactyloides* FL  
676 was aligned against *T. dactyloides* KS. Randomly sampled regions of the alignment were used as  
677 markers for input into ALLMAPS.

678 For nine individuals in the genus *Zea*, pan-genome anchor markers were used to further  
679 scaffold contigs or scaffolds to chromosomes, as in Hufford et al. (2021).

680



681 **Genome size estimation**

682 We estimated genome size of sequenced individuals outside the Tripsacinae using  
683 methods modified from (Doležel et al., 2007), and described in Phillips et al., (2023). Two  
684 internal standards were used, depending on the reference: maize B73 inbred line (5.16 pg/2C)  
685 and our *A. virginicum* accession (2.17 pg/2C). We placed approximately 10 × 1 cm of fresh leaf  
686 tissue for the target and sample standard in a plastic square petri dish, and added 1.25 mL of a  
687 chopping solution composed of 1 mL LB01 buffer solution, 250 µL propidium iodide (PI) stock (2  
688 mg/mL), and 25 µL RNase (1 mg/mL) (Doležel et al., 2007). We next chopped the tissue into 2–4  
689 mm lengths and mixed the chopping solution through the leaves by pipetting. The solution was  
690 then pipetted through a 30 µm sterile single-pack CellTrics filter into a 2 mL Rohren tube on ice.  
691 At least three replicates were chopped separately and analyzed for each individual. The  
692 samples were left to chill for 20 min before analysis with a BD Accuri C6 flow cytometer.  
693 Samples were run in Auto Collect mode with a 5-minute run limit, slow fluidics option, a  
694 forward scatter height (FSC-H) threshold with less than 200,000 events, and a one-cycle wash.  
695 The cell count, coefficient of variation of FL2-A, and mean FL2-A were recorded for the target  
696 and reference sample with no gating. Results were analyzed separately for each replicate and  
697 manually annotated to designate the set of events. We averaged values across all replicates of  
698 each individual (Supplemental Table S3).

699

700 **Chromosome counts**

701 Newly formed root tips were harvested from greenhouse-grown reference plants  
702 approximately one week after transplanting to new growth medium. Two or more roots were  
703 examined for each species. Exposure to nitrous oxide (160 psi for 2.5 - 3 hr) was used to stop  
704 mitosis in metaphase (Kato, 1999). Methods used for fixation, enzymatic digestion of  
705 meristematic tissue, and slide preparation have been described in detail (Kato et al., 2011;  
706 Phillips et al., 2023). The digestion times and amount of acetic acid-methanol solution used to  
707 resuspend the digested meristem varied based on meristem size. The cross-linked suspension  
708 was stained with a 1/20 dilution of Vectashield with 4',6-diamidino-2-phenylindole (DAPI)  
709 (Vector Laboratories, Burlingame, CA). Images were acquired with Applied Spectral Imaging  
710 (ASI) software (Carlsbad, CA) on an Olympus BX61 fluorescence microscope and saved in  
711 grayscale. The background was reduced using Adobe Photoshop Brightness/Contrast and/or  
712 Curves functions. Some images were sharpened with either ASI or Microsoft PowerPoint  
713 software.

714

715 **Gene annotation and homolog identification**

716 We used Helixer (Stiehler et al., 2021), a deep learning gene prediction model to  
717 produce annotations for each genome. We used the plant model, trained on 51 land plant  
718 genomes. As these models often generate false positive gene annotations, often including  
719 transposable elements in the gene set, we aimed to filter these annotations. We ran  
720 Orthofinder v2.5.5 (Emms & Kelly, 2019) within GENESPACE v1.3.1 (Lovell et al., 2022) on all  
721 assemblies and the *Paspalum vaginatum* outgroup. We retained orthogroups with >40 and  
722 <200 copies, generating an “orthology filtered gene set,” which we use for analyses involving  
723 gene copy number (detailed in *Gene Ontology searches* section).

724 We generated another set of “traditional” gene annotations using *ab initio* predictions  
725 from BRAKER (v2.1.6) (Brůna et al., 2021), direct evidence inferred from transcript assemblies  
726 using the BIND strategy (Li et al., 2022), and homology predictions using *Sorghum bicolor* and  
727 *Zea mays* subsp. *mays* (B73v5) annotations were generated using GeMoMa (v1.8) (Keilwagen et  
728 al., 2018) Annotation Filter tool. Predictions were prioritized using weights, with the highest for  
729 homology (1.0), followed by direct evidence (0.9), and the lowest for gene predictions from *ab*  
730 *initio* methods (0.1). Weights were assigned based on reliability, and the Annotation Filter  
731 ensured prediction completeness, external evidence support, and RNAseq support. The  
732 canonical transcript for each gene was predicted using TRaCE (Olson & Ware, 2021).

733 Traditional gene annotation pipelines can struggle in polyploid and allelic assemblies,  
734 due to multiple mapping across homologous copies. Additionally, the close gene spacing in  
735 some of our assemblies led to inappropriate merging of adjacent gene models, particularly in  
736 genomes less than 1 gigabase. We provide these traditional gene models for consistency with  
737 community standards, but for comparisons across taxa and analyses in this paper, we use the  
738 “orthology filtered gene set” derived from Helixer models and a set of syntenic anchor genes  
739 described below in analyses throughout this paper.

#### 740 **Repeat annotation**

741 We ran EDTA (Ou et al., 2019) with default parameters on each assembly, generating a  
742 repeat library and gff annotation. We used the “traditional” gene annotations as input to EDTA.  
743 As no gene annotation was produced for *Zea luxurians*, we supplied the B73v5 gene sequences  
744 for masking purposes. Although EDTA can be supplied with a reference TE library, such curated  
745 libraries are only available for maize. In order to compare TEs across our taxonomic sampling,  
746 we performed de novo searches on each assembly, so methods were consistent.

747 To calculate TE family evenness, we used Pielou’s evenness metric (Pielou, 1966),  
748 counting the number of copies in each TE family in each genome. Pielou’s evenness is a ratio  
749 between the Shannon Index and the hypothetical value if all families had the same relative  
750 abundance.

#### 751 **Tandem repeat identification and masking**

752 Initial investigation of EDTA outputs suggested many megabases of *Zea* knob sequences  
753 were annotated as different TE superfamilies in each assembly. To identify tandem repeats that  
754 may be falsely annotated as TEs, we used TRASH (Włodzimierz et al., 2023) with default  
755 parameters to annotate tandem repeats in each assembly. Within each assembly, we filtered to  
756 unique primary consensus sequences at least 40 bp long and found in at least five positions  
757 (discontinuous tandem arrays). We used these sequences as a repeat library to mask the  
758 assembly it was generated from, using RepeatMasker v.4.1.0 (Smit et al., 2013) with  
759 parameters (-q -no\_is -norna -nolow -div 40), generating gff output with (-gff). We merged this  
760 RepeatMasker output with the EDTA gff, first using bedtools subtract to remove EDTA TEs that  
761 overlapped tandem repeats, then concatenating this output with the tandem repeats. There  
762 was one remaining knob-related sequence incorporated into a Ty3 family that makes up ~200  
763 Mb of sequence in *Z. nicaraguensis* (TE\_00015576), which we removed for analyses of TE  
764 content.

765 To identify terminal telomere sequence, we searched contigs greater than 1 Mb for the  
766 Poales AAACCT telomere repeat using *tidk* (Brown et al., 2023). We consider the telomere  
767 present at a sequence end if >100 repeats are present in the terminal 30 kilobases.

### 768 **Gene synteny identification**

769 We used AnchorWave (Song et al., 2022) to generate syntenic paths through each  
770 assembly, with values informed by evidence of polyploidy (Supplemental Table 3). We used the  
771 haploid assembly and gene annotation of the non-Andropogoneae outgroup *Paspalum*  
772 *vaginatum* (v3.1; Sun et al., 2022) as the reference, and allowed each gene anchor to  
773 participate in up to 2 paths for diploids, 4 for tetraploids and paleotetraploids, and up to 6 for  
774 hexaploids. In cases of uncertain ploidy, we increased the number of paths allowed to 6. For  
775 each *Paspalum* gene, we counted how many paths it participated in per taxon, and recorded  
776 the start/end coordinates of each genic CDS alignment. We filtered to anchors present in at  
777 least one copy in 32/35 assemblies (>90%), to generate a set of 9,168 conserved syntenic  
778 anchors, which we refer to as our “syntenic gene anchors.”

779 Due to the extensive chromosome collinearity between *Paspalum* and Andropogoneae,  
780 we used these syntenic blocks to count chromosome rearrangements in each assembly. We  
781 used syntenic blocks containing at least 30 genes, and considered each contig that contained  
782 syntenic blocks that matched two *Paspalum* chromosomes as a rearrangement. We excluded  
783 four low-contiguity assemblies from these calculations (*Thelepogon elegans*, “*Andropogon*”  
784 *burmanicus*, *Rhytachne rottboelloides*, *Cymbopogon citratus*).

### 785 **Ploidy**

786 To estimate ploidy, we integrated cytology, gene synteny counts, heterozygosity, and  
787 literature reports. Historical conflict over the base chromosome number of Andropogoneae can  
788 complicate interpretations, as  $n=5$  and  $n=10$  can be indistinguishable when conflated with  
789 different ploidy levels. For example, a  $n=5$  tetraploid would have 20 pachytene chromosomes,  
790 as would a  $n=10$  diploid. Additionally, whether alleles are assembled into distinct contigs  
791 impacts the depth of syntenic blocks, as a tetraploid with alleles collapsed into two contigs  
792 would have a depth of 2, just as a diploid with both alleles assembled would. Using literature  
793 searches of chromosome counts and our own chromosome squashes as a guide to possible  
794 ploidy, we first assigned assemblies as allelic tetraploids or hexaploids if their synteny depth  
795 reached 4 or 6, and haploid hexaploids when the modal synteny depth was 3. As we measured  
796 1C genome size, an allelic assembly will have ~2 times the Mb of DNA as the flow cytometry  
797 measurement, allowing us to further classify tetraploid and diploid taxa. Our final assignments  
798 of ploidy are shown in Supplementary Table S3, and haploid assemblies are designated with a  
799 (\*) after their name in Figure 1. When presenting genome assembly size in Figure 1B, we divide  
800 allelic assemblies by two to present haploid size, and when presenting gene counts and repeat  
801 content throughout the paper, we divide values for allelic assemblies by two to present  
802 comparable haploid equivalents.

### 803 **Heterozygosity**

804 For taxa with haploid assemblies, we mapped raw reads back to the reference genome  
805 using *minimap2* (H. Li, 2018) with `-ax map-pb`, and called SNPs using *DeepVariant* 1.6.1 (Poplin  
806 et al., 2018). We used the number of heterozygous SNPs in the resulting *gvcf* divided by

807 homozygous reference calls in the same gvcf as a measurement of heterozygosity. This helped  
808 classify ploidy for two taxa with ambiguous assignment, *E. tripsacoides* and *R. tuberculosa*. As  
809 both these assemblies showed very low heterozygosity, we assigned *E. tripsacoides* as a haploid  
810 assembly of a tetraploid, and *R. tuberculosa* as a haploid assembly of a diploid. However, such a  
811 result could arise after many generations of selfing, which may be possible for congener  
812 *Rottboellia exalta* (Supplemental Text). We do not report heterozygosity values for *C.*  
813 *serrulatus*, as it appears to have elevated heterozygosity estimates arising from base errors of  
814 early generation Nanopore reads.

### 815 **Gene tree reconstruction**

816 For each *Paspalum* syntenic anchor, we extracted the corresponding genomic sequence  
817 from each assembly using samtools faidx (Danecek et al., 2021). From these syntenic anchors,  
818 we combined each sequence into a multiple-fasta of CDS and intronic sequence of each anchor  
819 gene, and aligned them with MAFFT (parameters --genafpair --maxiterate 1000 --  
820 adjustdirection) (Kato & Standley, 2013). We generated a gene tree from this multiple  
821 sequence alignment using RAxML (Stamatakis, 2014) with 100 rapid bootstrap replicates  
822 (parameters -m GTRGAMMA -p 12345 -x 12345 -# 100 -f a). As we included introns, some  
823 alignments failed to align due to memory issues, so we produced gene trees for 7,725 syntenic  
824 anchors. We note that this set of genes is not reliant on the gene annotation of each assembly,  
825 so it likely captures both genes and pseudogenes.

826 To calculate synonymous diversity between copies, we extracted codon positions based  
827 on the alignment and the *Paspalum* gene used as an anchor. We calculated all pairwise  
828 comparisons of all tips in the gene tree and filtered to gene copies within a polyploid to  
829 characterize intraspecific Ks values.

### 830 **Species tree reconstruction**

831 We adjusted the tip labels in each gene tree to be the species name, such that gene  
832 trees were multilabelled for allelic and homeologous copies, and provided these 7,725 gene  
833 trees as input to ASTRAL-PRO3 (Tabatabaee et al., 2023; Zhang et al., 2020) using default  
834 parameters.

### 835 **Gene Ontology searches**

836 We used Blast2GO (Conesa et al., 2005) to generate Gene Ontology categories for each  
837 Helixer gene in each species, then merged GO terms across all copies within an orthogroup. We  
838 tested for enrichment using TopGO (Alexa & Rahnenführer, 2009). We identified a set of  
839 orthogroups with copy number deviations from the standardized genome count for polyploids  
840 vs diploids. To determine whether gene copy number deviated between the groups, we first  
841 calculated the median copy number for each gene within each assembly. We then standardized  
842 the assembly's copy number by subtracting this median. For each orthogroup, we used a  
843 Wilcoxon rank-sum test to compare deviations in copy number between the groups. P-values  
844 were obtained for each orthogroup-specific comparison, and ranked to select the top 100  
845 genes for exploration with GO via TopGO.

### 846 **Subgenome phasing**

847 We ran SubPhaser (Jia et al., 2022), with a k-mer length of 17 and a minimum k-mer  
848 count of 200. Homologous sequences were defined based on the AnchorWave syntenic regions.

849

850 ***Conservation and acceleration of genomic elements at different phylogenetic scales and***  
851 ***ploidies***

852 Andropogoneae genomes soft-masked for EDTA repeats were aligned with Cactus 2.1.1  
853 (Armstrong et al., 2020) and a multiple alignment based on the B73 reference was extracted  
854 from the alignment graph. Syntenic alignments to B73 were retained based on MCScanX (Y.  
855 Wang et al., 2012) syntenic gene blocks using traditional gene annotations. No gene  
856 annotations were generated to annotate the *Zea luxurians* genome, so the annotation used for  
857 MCScanX was generated by lifting over the *Z. nicaraguensis* annotation using LiftOff 1.6.2  
858 (Shumate & Salzberg, 2021) with the “polish” flag. Chimeric subgenomes were assigned for all  
859 polyploid species based on MCScanX synteny to the ancestral *Paspalum vaginatum* genome,  
860 clustering scaffolds syntenic to each *P. vaginatum* chromosome into subgenomes based on a  
861 custom greedy algorithm that minimized overlap between subgenomes. The resulting  
862 subgenomes are chimeric as each chromosome may be composed of different biological  
863 subgenomes. We do not expect this to impact our analysis of conserved elements as divergence  
864 between subgenomes was relatively low.

865 A neutral model of evolution was fit to the Andropogoneae phylogeny using fourfold  
866 degenerate sites from maize chromosome 10 with phyloFit from the PHAST 1.4 package (Hubisz  
867 et al., 2011). A set of most conserved elements was generated using the PhastCons “most-  
868 conserved” flag from the PHAST package with an expected length of 8bp, after training to  
869 generate models of conserved and non-conserved elements using genome-wide multiple  
870 alignments with “--coverage 0.45”. To prevent reference-bias in the discovery of CNS, the B73  
871 reference was masked and all other Tripsacinae were excluded for the phastCons analyses. The  
872 resulting 2,302,710 conserved elements were then filtered to exclude elements shorter than 5  
873 bp or with  $\geq 1$  bp overlap with CDS, introns, or untranslated regions (UTR). In addition,  
874 conserved elements with BLASTX hits with e-value  $\leq 0.01$  to the Swissprot Viridiplantae  
875 protein database were removed, to ensure unannotated genes and pseudogenes were  
876 excluded.

877 To determine the presence of a CNS element in each Andropogoneae genome, we  
878 required an alignment covering at least 50% of the element, excluding gaps. CNS were classified  
879 as “downstream”, “upstream”, “downstream distal”, or “upstream distal” based on the B73  
880 gene annotation and a threshold of 1 kbp distance from the nearest gene feature to determine  
881 whether a CNS was distal. Fold enrichment of genomic features in the conserved elements was  
882 calculated following Song et al., (2021) by dividing the proportion of bp of a feature that were  
883 conserved by the proportion of bp in the genome that overlap the feature. Chromatin loops  
884 were determined based on HiC data (Ricci et al., 2019), and accessible chromatin regions (ACR)  
885 were based on single-cell ATAC experiments from (Marand et al., 2021). MNase-defined  
886 cistrome-Occupancy Analysis motifs were obtained from Savadel et al., (2021) and transcription  
887 factor bound regions were based on ChIP-seq provided by Tu et al., (2020). Other genomic  
888 annotations including of TEs and noncoding RNA were based on the MaizeGDB B73v5 genome  
889 annotation. We used phyloP with “--method LRT --mode ACC” from the PHAST package to test  
890 for lineage-specific acceleration in Tripsacinae in elements conserved across Andropogoneae.  
891 Multiple testing correction of LRT p-values was conducted using the Benjamini-Hochberg

892 method with an FDR threshold of 0.05. A GO enrichment analysis of all CNS and Tripsacinae-  
893 accelerated CNS was conducted using rGREAT 1.1.0 (Gu & Hübschmann, 2023) using the B73 v5  
894 annotation and default parameters. For Tripsacinae-accelerated CNS the CNS was used as the  
895 background. GO terms with a fold enrichment <2 and an adjusted p-value  $\geq 0.1$  were filtered.

896 We assessed the turnover of TFBS across Andropogoneae based on alignments to B73  
897 predicted TFBS in the multiple alignment as well as predicted TFBS for each genome. TFBS were  
898 predicted based on the 46 representative plant motifs in the JASPAR 2022 plant-specific  
899 database (Castro-Mondragon et al., 2022), which were trimmed using universal motif 4.3  
900 (Tremblay, 2024) with a minimum allowed information content of 0.5 bits. Motif scanning was  
901 conducted using a custom kotlin script with a detection threshold of 70% of the maximum  
902 position weight matrix score. To focus on the potentially most functionally relevant TFBS, we  
903 used TFBS in the 1kb region upstream of the translation start sites of B73 genes, which also met  
904 the following criteria: has gene expression >0 TPM (Hufford et al., 2021), is a core gene across  
905 maize NAM lines (Hufford et al., 2021), is not a tandem duplicate in B73, the 1kb upstream of  
906 the translation start site intersects with  $\geq 1$  ChIP sequencing peak from data generated by Tu et  
907 al. (2020), and has  $\geq 1$  syntenic collinear ortholog across the other Andropogoneae species. For  
908 each Andropogoneae query species, we compared the TFBS in the 1kb region upstream of the  
909 translation start sites of the selected B73 genes and the TFBS in the 1kb region upstream of the  
910 translation start sites of the collinear gene in the query species, selecting a single random  
911 ortholog if there were multiple orthologous collinear genes in the query species. A single  
912 representative subgenome for each polyploid query species was used. To account for  
913 limitations of the alignment and structural variation, a reference TFBS was considered present  
914 in a query species if it was aligned and a matching prediction was present or if it was not  
915 aligned but a matching prediction was present in the query region. To compare the TFBS  
916 turnover, genic turnover was also calculated using the Orthofinder orthogroups for the same  
917 set of genes analyzed for TFBS turnover, excluding orthogroups with multi-copy B73 genes. *Z.*  
918 *nicaraguensis* was excluded from these analyses because its gene annotation was incomplete  
919 due to missing sequences in the main haplotype assembly, and *Z. luxurians* was excluded  
920 because it did not have a high-quality gene annotation. To compare the turnover rates with the  
921 phylogenetic distance to *Z. mays*, sequence divergence to each species was calculated using the  
922 set of fourfold degenerate sites used to calculate the neutral model.  
923

## 924 **Data Availability**

925 Raw data will be available under NCBI/EBI BioProject PRJEB50280 and genome  
926 assemblies at USDA Ag Data Commons upon publication. The code used to generate assemblies  
927 and conserved noncoding sequence analyses is available at  
928 [https://github.com/HuffordLab/panand\\_genome\\_evolution](https://github.com/HuffordLab/panand_genome_evolution), and code for other analyses and  
929 figures at [https://github.com/mcstitzer/panand\\_assemblies](https://github.com/mcstitzer/panand_assemblies). Additionally, figures highlighting  
930 each plant can be viewed at [https://mcstitzer.github.io/panand\\_assemblies/](https://mcstitzer.github.io/panand_assemblies/)

## 931 **Acknowledgements**

933 This article is based upon work supported by the National Science Foundation under  
934 Grant Number 1822330, and the U.S. Department of Agriculture–Agricultural Research Service  
935 (USDA-ARS) under Project Number 5030-21000-072-00-D (Corn Insects and Crop Genetics  
936 Research Unit, Ames, Iowa), Project Number 6066-21310-005-00-D (Genomics and  
937 Bioinformatics Research Unit, Stoneville, MS), and Project Number 8062-21000-043-00-D  
938 (Plant, Soil and Nutrition Research Unit, Ithaca, NY). Mention of trade names or commercial  
939 products in this publication is solely for the purpose of providing specific information and does  
940 not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an  
941 equal opportunity provider and employer.

942 A.S. and A.C.S were supported by the National Institute of General Medical Sciences,  
943 National Institutes of Health, under award number R01GM102192. M.C.S. was supported by  
944 the National Science Foundation Postdoctoral Research Fellowship in Biology under Grant  
945 Number 1907343.

946 This research used resources provided by the SCINet project and the AI Center of  
947 Excellence of the USDA Agricultural Research Service, ARS project numbers 0201-88888-003-  
948 000D and 0201-88888-002-000D and The Texas Advanced Computing Center (TACC) at The  
949 University of Texas at Austin. Computational resources and data management were provided  
950 by the Bioinformatics Facility (RRID:SCR\_021757) at the Cornell Institute of Biotechnology. The  
951 authors acknowledge financial support from Inari Agriculture for the sequencing of six species  
952 included in this project, and financial support from Corteva Agriscience for sequencing of ten  
953 individuals.

954 Lynn Clark (Iowa State University) provided germplasm for *Pogonatherum paniceum*.  
955 We acknowledge all who have contributed to the conservation, cultivation, and study of the  
956 natural diversity of Andropogoneae grasses.

957

## 958 **References**

959

- 960 Alexa, A., & Rahnenführer, J. (2009). Gene set enrichment analysis with topGO. *Bioconductor Improv*, 27,  
961 1–26.
- 962 Alix, K., Gérard, P. R., Schwarzacher, T., & Heslop-Harrison, J. S. (Pat). (2017). Polyploidy and interspecific  
963 hybridization: Partners for adaptation, speciation and evolution in plants. *Annals of Botany*,  
964 120(2), 183–194. <https://doi.org/10.1093/aob/mcx079>
- 965 Armstrong, J., Hickey, G., Diekhans, M., Fiddes, I. T., Novak, A. M., Deran, A., Fang, Q., Xie, D., Feng, S.,  
966 Stiller, J., Genereux, D., Johnson, J., Marinescu, V. D., Alföldi, J., Harris, R. S., Lindblad-Toh, K.,  
967 Haussler, D., Karlsson, E., Jarvis, E. D., ... Paten, B. (2020). Progressive Cactus is a multiple-  
968 genome aligner for the thousand-genome era. *Nature*, 587(7833), 246–251.  
969 <https://doi.org/10.1038/s41586-020-2871-y>
- 970 AuBuchon-Elder, T., Minx, P., Bookout, B., & Kellogg, E. A. (2023). Plant conservation assessment at  
971 scale: Rapid triage of extinction risks. *PLANTS, PEOPLE, PLANET*, 5(3), 386–397.  
972 <https://doi.org/10.1002/ppp3.10355>

- 973 Baduel, P., Bray, S., Vallejo-Marin, M., Kolář, F., & Yant, L. (2018). The “Polyploid Hop”: Shifting  
974 Challenges and Opportunities Over the Evolutionary Lifespan of Genome Duplications. *Frontiers*  
975 *in Ecology and Evolution*, 6. <https://doi.org/10.3389/fevo.2018.00117>
- 976 Baduel, P., Quadrana, L., Hunter, B., Bomblies, K., & Colot, V. (2019). Relaxed purifying selection in  
977 autopolyploids drives transposable element over-accumulation which provides variants for local  
978 adaptation. *Nature Communications*, 10(1), 5818. <https://doi.org/10.1038/s41467-019-13730-0>
- 979 Barakat, A., Szick-Miranda, K., Chang, I.-F., Guyot, R., Blanc, G., Cooke, R., Delseny, M., & Bailey-Serres, J.  
980 (2001). The Organization of Cytoplasmic Ribosomal Protein Genes in the Arabidopsis Genome.  
981 *Plant Physiology*, 127(2), 398–415. <https://doi.org/10.1104/pp.010265>
- 982 Bennetzen, J. L., & Kellogg, E. A. (1997). Do Plants Have a One-Way Ticket to Genomic Obesity? *The Plant*  
983 *Cell*, 9(9), 1509–1514. <https://doi.org/10.1105/tpc.9.9.1509>
- 984 Bennetzen, J. L., Ma, J., & Devos, K. M. (2005). Mechanisms of Recent Genome Size Variation in  
985 Flowering Plants. *Annals of Botany*, 95(1), 127–132. <https://doi.org/10.1093/aob/mci008>
- 986 Bianconi, M. E., Hackel, J., Vorontsova, M. S., Alberti, A., Arthan, W., Burke, S. V., Duvall, M. R., Kellogg,  
987 E. A., Lavergne, S., McKain, M. R., Meunier, A., Osborne, C. P., Traiperm, P., Christin, P.-A., &  
988 Besnard, G. (2020). Continued Adaptation of C4 Photosynthesis After an Initial Burst of Changes  
989 in the Andropogoneae Grasses. *Systematic Biology*, 69(3), 445–461.  
990 <https://doi.org/10.1093/sysbio/syz066>
- 991 Bingham, E. T. (1980). Maximizing Heterozygosity in Autopolyploids. In W. H. Lewis (Ed.), *Polyploidy:*  
992 *Biological Relevance* (pp. 471–489). Springer US. [https://doi.org/10.1007/978-1-4613-3069-1\\_24](https://doi.org/10.1007/978-1-4613-3069-1_24)
- 993 Birchler, J. A., & Veitia, R. A. (2012). Gene balance hypothesis: Connecting issues of dosage sensitivity  
994 across biological disciplines. *Proceedings of the National Academy of Sciences*, 109(37), 14746–  
995 14753.
- 996 Blanc, G., & Wolfe, K. H. (2004). Widespread paleopolyploidy in model plant species inferred from age  
997 distributions of duplicate genes. *The Plant Cell*, 16(7), 1667–1678.
- 998 Bowers, J. E., & Paterson, A. H. (2021). Chromosome number is key to longevity of polyploid lineages.  
999 *New Phytologist*, 231(1), 19–28.
- 1000 Braz, G. T., do Vale Martins, L., Zhang, T., Albert, P. S., Birchler, J. A., & Jiang, J. (2020). A universal  
1001 chromosome identification system for maize and wild Zea species. *Chromosome Research*, 28,  
1002 183–194.
- 1003 Brown, M., González De la Rosa, P. M., & Mark, B. (2023). *A Telomere Identification Toolkit* (Version  
1004 v0.2.41) [Computer software]. Zenodo. <https://doi.org/10.5281/zenodo.10091385>
- 1005 Brůna, T., Hoff, K. J., Lomsadze, A., Stanke, M., & Borodovsky, M. (2021). BRAKER2: Automatic  
1006 eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein  
1007 database. *NAR Genomics and Bioinformatics*, 3(1), lqaa108.  
1008 <https://doi.org/10.1093/nargab/lqaa108>
- 1009 Buckler IV, E. S., Phelps-Durr, T. L., Buckler, C. S. K., Dawe, R. K., Doebley, J. F., & Holtsford, T. P. (1999).  
1010 Meiotic drive of chromosomal knobs reshaped the maize genome. *Genetics*, 153(1), 415–426.
- 1011 Buggs, R. J. A., Chamala, S., Wu, W., Tate, J. A., Schnable, P. S., Soltis, D. E., Soltis, P. S., & Barbazuk, W. B.  
1012 (2012). Rapid, Repeated, and Clustered Loss of Duplicate Genes in Allopolyploid Plant  
1013 Populations of Independent Origin. *Current Biology*, 22(3), 248–252.  
1014 <https://doi.org/10.1016/j.cub.2011.12.027>
- 1015 Burgess, D., & Freeling, M. (2014). The Most Deeply Conserved Noncoding Sequences in Plants Serve  
1016 Similar Functions to Those in Vertebrates Despite Large Differences in Evolutionary Rates. *The*  
1017 *Plant Cell*, 26(3), 946–961. <https://doi.org/10.1105/tpc.113.121905>
- 1018 Burns, R., Mandáková, T., Gunis, J., Soto-Jiménez, L. M., Liu, C., Lysak, M. A., Novikova, P. Y., & Nordborg,  
1019 M. (2021). Gradual evolution of allopolyploidy in Arabidopsis suecica. *Nature Ecology &*  
1020 *Evolution*, 5(10), 1367–1381. <https://doi.org/10.1038/s41559-021-01525-w>



- 1021 Campbell, C. S. (1982). Cleistogamy in *Andropogon* L. (Gramineae). *American Journal of Botany*, 69(10),  
1022 1625–1635. <https://doi.org/10.2307/2442917>
- 1023 Carino, D. A., & Daehler, C. C. (1999). Genetic variation in an apomictic grass, *Heteropogon contortus*, in  
1024 the Hawaiian Islands. *Molecular Ecology*, 8(12), 2127–2132. <https://doi.org/10.1046/j.1365-294x.1999.00786.x>
- 1026 Castro-Mondragon, J. A., Riudavets-Puig, R., Rauluseviciute, I., Berhanu Lemma, R., Turchi, L., Blanc-  
1027 Mathieu, R., Lucas, J., Boddie, P., Khan, A., Manosalva Pérez, N., Fornes, O., Leung, T. Y., Aguirre,  
1028 A., Hammal, F., Schmelter, D., Baranasic, D., Ballester, B., Sandelin, A., Lenhard, B., ... Mathelier,  
1029 A. (2022). JASPAR 2022: The 9th release of the open-access database of transcription factor  
1030 binding profiles. *Nucleic Acids Research*, 50(D1), D165–D173.  
1031 <https://doi.org/10.1093/nar/gkab1113>
- 1032 Celarier, R. P. (1957). *Elyonurus argenteus*, a South African grass with five chromosome pairs. *Bulletin of*  
1033 *the Torrey Botanical Club*, 157–162.
- 1034 Chen, J., Lu, L., Robb, S. M. C., Collin, M., Okumoto, Y., Stajich, J. E., & Wessler, S. R. (2020). Genomic  
1035 diversity generated by a transposable element burst in a rice recombinant inbred population.  
1036 *Proceedings of the National Academy of Sciences*, 117(42), 26288–26297.  
1037 <https://doi.org/10.1073/pnas.2015736117>
- 1038 Chen, L., Luo, J., Jin, M., Yang, N., Liu, X., Peng, Y., Li, W., Phillips, A., Cameron, B., Bernal, J. S., Rellán-  
1039 Álvarez, R., Sawers, R. J. H., Liu, Q., Yin, Y., Ye, X., Yan, J., Zhang, Q., Zhang, X., Wu, S., ... Yan, J.  
1040 (2022). Genome sequencing reveals evidence of adaptive variation in the genus *Zea*. *Nature*  
1041 *Genetics*, 54(11), 1736–1745. <https://doi.org/10.1038/s41588-022-01184-y>
- 1042 Cheng, H., Concepcion, G. T., Feng, X., Zhang, H., & Li, H. (2021). Haplotype-resolved de novo assembly  
1043 using phased assembly graphs with hifiasm. *Nature Methods*, 18(2), 170–175.
- 1044 Chin, C.-S., Peluso, P., Sedlazeck, F. J., Nattestad, M., Concepcion, G. T., Clum, A., Dunn, C., O'Malley, R.,  
1045 Figueroa-Balderas, R., Morales-Cruz, A., Cramer, G. R., Delledonne, M., Luo, C., Ecker, J. R.,  
1046 Cantu, D., Rank, D. R., & Schatz, M. C. (2016). Phased diploid genome assembly with single-  
1047 molecule real-time sequencing. *Nature Methods*, 13(12), 1050–1054.  
1048 <https://doi.org/10.1038/nmeth.4035>
- 1049 Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, 6(11),  
1050 836–846. <https://doi.org/10.1038/nrg1711>
- 1051 Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: A universal  
1052 tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*,  
1053 21(18), 3674–3676. <https://doi.org/10.1093/bioinformatics/bti610>
- 1054 Corneillie, S., De Storme, N., Van Acker, R., Fangel, J. U., De Bruyne, M., De Rycke, R., Geelen, D., Willats,  
1055 W. G. T., Vanholme, B., & Boerjan, W. (2019). Polyploidy Affects Plant Growth and Alters Cell  
1056 Wall Composition. *Plant Physiology*, 179(1), 74–87. <https://doi.org/10.1104/pp.18.00967>
- 1057 Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T.,  
1058 McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools.  
1059 *GigaScience*, 10(2), giab008. <https://doi.org/10.1093/gigascience/giab008>
- 1060 Dawe, R. K., Lowry, E. G., Gent, J. I., Stitzer, M. C., Swentowsky, K. W., Higgins, D. M., Ross-Ibarra, J.,  
1061 Wallace, J. G., Kanizay, L. B., Alabady, M., Qiu, W., Tseng, K.-F., Wang, N., Gao, Z., Birchler, J. A.,  
1062 Harkess, A. E., Hodges, A. L., & Hiatt, E. N. (2018). A Kinesin-14 Motor Activates Neocentromeres  
1063 to Promote Meiotic Drive in Maize. *Cell*, 173(4), 839–850.e18.  
1064 <https://doi.org/10.1016/j.cell.2018.03.009>
- 1065 De Smet, R., Adams, K. L., Vandepoele, K., Van Montagu, M. C., Maere, S., & Van de Peer, Y. (2013).  
1066 Convergent gene loss following gene and genome duplications creates single-copy families in  
1067 flowering plants. *Proceedings of the National Academy of Sciences*, 110(8), 2898–2903.
- 1068 Doebley, J. (1990). Molecular Evidence and the Evolution of Maize. *Economic Botany*, 44(3), 6–27.

- 1069 Doležel, J., Greilhuber, J., & Suda, J. (2007). Estimation of nuclear DNA content in plants using flow  
1070 cytometry. *Nature Protocols*, 2(9), 2233–2244.
- 1071 Doyle, J. J., & Coate, J. E. (2019). Polyploidy, the Nucleotype, and Novelty: The Impact of Genome  
1072 Doubling on the Biology of the Cell. *International Journal of Plant Sciences*, 180(1), 1–52.  
1073 <https://doi.org/10.1086/700636>
- 1074 Doyle, J. J., & Egan, A. N. (2010). Dating the origins of polyploidy events. *New Phytologist*, 186(1), 73–85.  
1075 <https://doi.org/10.1111/j.1469-8137.2009.03118.x>
- 1076 Dudchenko, O., Shamim, M. S., Batra, S. S., Durand, N. C., Musial, N. T., Mostofa, R., Pham, M., Hilaire, B.  
1077 G. S., Yao, W., Stamenova, E., Hoeger, M., Nyquist, S. K., Korchina, V., Pletch, K., Flanagan, J. P.,  
1078 Tomaszewicz, A., McAloose, D., Estrada, C. P., Novak, B. J., ... Aiden, E. L. (2018). *The Juicebox*  
1079 *Assembly Tools module facilitates de novo assembly of mammalian genomes with chromosome-*  
1080 *length scaffolds for under \$1000* (p. 254797). bioRxiv. <https://doi.org/10.1101/254797>
- 1081 Durand, N. C., Shamim, M. S., Machol, I., Rao, S. S. P., Huntley, M. H., Lander, E. S., & Aiden, E. L. (2016).  
1082 Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments. *Cell*  
1083 *Systems*, 3(1), 95–98. <https://doi.org/10.1016/j.cels.2016.07.002>
- 1084 Duvick, D. N. (2001). Biotechnology in the 1930s: The development of hybrid maize. *Nature Reviews*  
1085 *Genetics*, 2(1), 69–74. <https://doi.org/10.1038/35047587>
- 1086 Ebadi, M., Bafort, Q., Mizrachi, E., Audenaert, P., Simoens, P., Van Montagu, M., Bonte, D., & Van de  
1087 Peer, Y. (2023). The duplication of genomes and genetic networks and its potential for  
1088 evolutionary adaptation and survival during environmental turmoil. *Proceedings of the National*  
1089 *Academy of Sciences*, 120(41), e2307289120. <https://doi.org/10.1073/pnas.2307289120>
- 1090 Edger, P. P., & Pires, J. C. (2009). Gene and genome duplications: The impact of dosage-sensitivity on the  
1091 fate of nuclear genes. *Chromosome Research*, 17, 699–717.
- 1092 Emery, W. H. P., & Brown, W. V. (1958). Apomixis in the Gramineae. Tribe Andropogoneae:  
1093 Heteropogon Contortus. *Madroño*, 14(7), 238–246.
- 1094 Emms, D. M., & Kelly, S. (2019). OrthoFinder: Phylogenetic orthology inference for comparative  
1095 genomics. *Genome Biology*, 20, 1–14.
- 1096 Engelhorn, J., Snodgrass, S. J., Kok, A., Seetharam, A. S., Schneider, M., Kiwit, T., Singh, A., Banf, M.,  
1097 Khaipho-Burch, M., Runcie, D. E., Sanchez-Camargo, V. A., Torres-Rodriguez, J. V., Sun, G., Stam,  
1098 M., Fiorani, F., Beier, S., Schnable, J. C., Bass, H. W., Hufford, M. B., ... Hartwig, T. (2024). *Genetic*  
1099 *variation at transcription factor binding sites largely explains phenotypic heritability in maize* (p.  
1100 2023.08.08.551183). bioRxiv. <https://doi.org/10.1101/2023.08.08.551183>
- 1101 Estep, M. C., McKain, M. R., Vela Diaz, D., Zhong, J., Hodge, J. G., Hodkinson, T. R., Layton, D. J.,  
1102 Malcomber, S. T., Pasquet, R., & Kellogg, E. A. (2014). Allopolyploidy, diversification, and the  
1103 Miocene grassland expansion. *Proceedings of the National Academy of Sciences*, 111(42),  
1104 15149–15154. <https://doi.org/10.1073/pnas.1404177111>
- 1105 Fedoroff, N. V. (2012). Transposable Elements, Epigenetics, and Genome Evolution. *Science*, 338(6108),  
1106 758–767. <https://doi.org/10.1126/science.338.6108.758>
- 1107 Feuillet, C., & Keller, B. (1999). High gene density is conserved at syntenic loci of small and large grass  
1108 genomes. *Proceedings of the National Academy of Sciences of the United States of America*,  
1109 96(14), 8265–8270. <https://doi.org/10.1073/pnas.96.14.8265>
- 1110 Freeling, M. (2009). Bias in plant gene content following different sorts of duplication: Tandem, whole-  
1111 genome, segmental, or by transposition. *Annual Review of Plant Biology*, 60(1), 433–453.
- 1112 Fu, H., & Dooner, H. K. (2002). Intraspecific violation of genetic colinearity and its implications in maize.  
1113 *Proceedings of the National Academy of Sciences of the United States of America*, 99(14), 9573–  
1114 9578. <https://doi.org/10.1073/pnas.132259199>
- 1115 Gaut, B. S., Morton, B. R., McCaig, B. C., & Clegg, M. T. (1996). Substitution rate comparisons between  
1116 grasses and palms: Synonymous rate differences at the nuclear gene Adh parallel rate

- 1117 differences at the plastid gene *rbcl*. *Proceedings of the National Academy of Sciences*, 93(19),  
1118 10274–10279. <https://doi.org/10.1073/pnas.93.19.10274>
- 1119 Ghannoum, O., Evans, J. R., & von Caemmerer, S. (2011). Chapter 8 Nitrogen and Water Use Efficiency of  
1120 C4 Plants. In A. S. Raghavendra & R. F. Sage (Eds.), *C4 Photosynthesis and Related CO2*  
1121 *Concentrating Mechanisms* (pp. 129–146). Springer Netherlands. [https://doi.org/10.1007/978-](https://doi.org/10.1007/978-90-481-9407-0_8)  
1122 [90-481-9407-0\\_8](https://doi.org/10.1007/978-90-481-9407-0_8)
- 1123 Gibson, D. J. (2009). *Grasses and Grassland Ecology*. Oxford University Press.
- 1124 Grass Phylogeny Working Group III. (2024). A nuclear phylogenomic tree of grasses (Poaceae) recovers  
1125 current classification despite gene tree incongruence. *New Phytologist*, 245(2), 818–834.
- 1126 Gu, Z., & Hübschmann, D. (2023). rGREAT: An R/bioconductor package for functional enrichment on  
1127 genomic regions. *Bioinformatics*, 39(1), btac745.  
1128 <https://doi.org/10.1093/bioinformatics/btac745>
- 1129 Gupta, R. C., Gupta, A., & Kaur, N. (2017). Meiotic studies in some members of tribe Andropogoneae  
1130 (Poaceae) from semi desert area of North India. *Cytologia*, 82(2), 105–113.
- 1131 Haldane, J. (1933). The part played by recurrent mutation in evolution. *The American Naturalist*,  
1132 67(708), 5–19.
- 1133 Han, Y., Xin, M., Huang, K., Xu, Y., Liu, Z., Hu, Z., Yao, Y., Peng, H., Ni, Z., & Sun, Q. (2016). Altered  
1134 expression of Ta4 gene by genome interplay shapes root hair length in allopolyploid wheat. *New*  
1135 *Phytologist*, 209(2), 721–732. <https://doi.org/10.1111/nph.13615>
- 1136 Haudry, A., Platts, A. E., Vello, E., Hoen, D. R., Leclercq, M., Williamson, R. J., Forczek, E., Joly-Lopez, Z.,  
1137 Steffen, J. G., Hazzouri, K. M., Dewar, K., Stinchcombe, J. R., Schoen, D. J., Wang, X., Schmutz, J.,  
1138 Town, C. D., Edger, P. P., Pires, J. C., Schumaker, K. S., ... Blanchette, M. (2013). An atlas of over  
1139 90,000 conserved noncoding sequences provides insight into crucifer regulatory regions. *Nature*  
1140 *Genetics*, 45(8), 891–898. <https://doi.org/10.1038/ng.2684>
- 1141 Hawkins, J. S., Kim, H., Nason, J. D., Wing, R. A., & Wendel, J. F. (2006). Differential lineage-specific  
1142 amplification of transposable elements is responsible for genome size variation in *Gossypium*.  
1143 *Genome Research*, 16(10), 1252–1261. <https://doi.org/10.1101/gr.5282906>
- 1144 Hirsch, C. D., & Springer, N. M. (2017). Transposable element influences on gene expression in plants.  
1145 *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms*, 1860(1), 157–165.  
1146 <https://doi.org/10.1016/j.bbagr.2016.05.010>
- 1147 Hubisz, M. J., Pollard, K. S., & Siepel, A. (2011). PHAST and RPHAST: Phylogenetic analysis with  
1148 space/time models. *Briefings in Bioinformatics*, 12(1), 41–51.  
1149 <https://doi.org/10.1093/bib/bbq072>
- 1150 Hufford, M. B., Seetharam, A. S., Woodhouse, M. R., Chougule, K. M., Ou, S., Liu, J., Ricci, W. A., Guo, T.,  
1151 Olson, A., Qiu, Y., Della Coletta, R., Tittes, S., Hudson, A. I., Marand, A. P., Wei, S., Lu, Z., Wang,  
1152 B., Tello-Ruiz, M. K., Piri, R. D., ... Dawe, R. K. (2021). De novo assembly, annotation, and  
1153 comparative analysis of 26 diverse maize genomes. *Science*, 373(6555), 655–662.  
1154 <https://doi.org/10.1126/science.abg5289>
- 1155 Ickert-Bond, S. M., Sousa, A., Min, Y., Loera, I., Metzgar, J., Pellicer, J., Hidalgo, O., & Leitch, I. J. (2020).  
1156 Polyploidy in gymnosperms – Insights into the genomic and evolutionary consequences of  
1157 polyploidy in *Ephedra*. *Molecular Phylogenetics and Evolution*, 147, 106786.  
1158 <https://doi.org/10.1016/j.ympev.2020.106786>
- 1159 Jia, K.-H., Wang, Z.-X., Wang, L., Li, G.-Y., Zhang, W., Wang, X.-L., Xu, F.-J., Jiao, S.-Q., Zhou, S.-S., Liu, H.,  
1160 & others. (2022). SubPhaser: A robust allopolyploid subgenome phasing method based on  
1161 subgenome-specific k-mers. *New Phytologist*, 235(2), 801–809.
- 1162 Kato, A. (1999). Air drying method using nitrous oxide for chromosome counting in maize. *Biotechnic &*  
1163 *Histochemistry: Official Publication of the Biological Stain Commission*, 74(3), 160–166.  
1164 <https://doi.org/10.3109/10520299909047968>

- 1165 Kato, A., Lamb, J. C., Albert, P. S., Danilova, T., Han, F., Gao, Z., Findley, S., & Birchler, J. A. (2011).  
1166 Chromosome painting for plant biotechnology. *Methods in Molecular Biology (Clifton, N.J.)*, 701,  
1167 67–96. [https://doi.org/10.1007/978-1-61737-957-4\\_4](https://doi.org/10.1007/978-1-61737-957-4_4)
- 1168 Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7:  
1169 Improvements in performance and usability. *Molecular Biology and Evolution*, 30(4), 772–780.  
1170 <https://doi.org/10.1093/molbev/mst010>
- 1171 Keilwagen, J., Hartung, F., Paulini, M., Twardziok, S. O., & Grau, J. (2018). Combining RNA-seq data and  
1172 homology-based gene prediction for plants, animals and fungi. *BMC Bioinformatics*, 19, 1–12.
- 1173 Kellogg, E. A. (2015). Tribe Andropogoneae Dumort. In *The families and genera of vascular plants* (pp.  
1174 289–314).  
1175 [https://scholar.google.com/scholar\\_lookup?title=Tribe+Andropogoneae+Dumort.&author=E.+A.+Kellogg&volume=XIII&publication\\_year=2015&pages=289-314](https://scholar.google.com/scholar_lookup?title=Tribe+Andropogoneae+Dumort.&author=E.+A.+Kellogg&volume=XIII&publication_year=2015&pages=289-314)
- 1176 Kellogg, E. A. (2016). Has the connection between polyploidy and diversification actually been tested?  
1177 *Current Opinion in Plant Biology*, 30, 25–32. <https://doi.org/10.1016/j.pbi.2016.01.002>
- 1178 Kellogg, E. A., & Bennetzen, J. L. (2004). The evolution of nuclear genome structure in seed plants.  
1179 *American Journal of Botany*, 91(10), 1709–1725.
- 1180  
1181 Kempton, J. H., & Popenoe, W. (1937). *Teosinte in Guatemala: Report of an Expedition to Guatemala, El*  
1182 *Salvador, and Chiapas, Mexico*. Carnegie Institution of Washington.
- 1183 Kolář, F., Čertner, M., Suda, J., Schönswetter, P., & Husband, B. C. (2017). Mixed-Ploidy Species: Progress  
1184 and Opportunities in Polyploid Research. *Trends in Plant Science*, 22(12), 1041–1055.  
1185 <https://doi.org/10.1016/j.tplants.2017.09.011>
- 1186 Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., & Phillippy, A. M. (2017). Canu: Scalable  
1187 and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome*  
1188 *Research*, 27(5), 722–736. <https://doi.org/10.1101/gr.215087.116>
- 1189 Laurie, D. A., & Bennett, M. D. (1985). Nuclear DNA content in the genera Zea and Sorghum.  
1190 Intergeneric, interspecific and intraspecific variation. *Heredity*, 55(3), 307–313.  
1191 <https://doi.org/10.1038/hdy.1985.112>
- 1192 Lee, M., & Phillips, R. (1987). Genomic rearrangements in maize induced by tissue culture. *Genome*,  
1193 29(1), 122–128.
- 1194 Lehmann, C. E. R., Griffith, D. M., Simpson, K. J., Anderson, T. M., Archibald, S., Beerling, D. J., Bond, W.  
1195 J., Denton, E., Edwards, E. J., Forrestel, E. J., Fox, D. L., Georges, D., Hoffmann, W. A., Kluyver, T.,  
1196 Mucina, L., Pau, S., Ratnam, J., Salamin, N., Santini, B., ... Osborne, C. P. (2019). *Functional*  
1197 *diversification enabled grassy biomes to fill global climate space* (p. 583625). bioRxiv.  
1198 <https://doi.org/10.1101/583625>
- 1199 Leitch, A. R., & Leitch, I. J. (2008). Genomic Plasticity and the Diversity of Polyploid Plants. *Science*,  
1200 320(5875), 481–483. <https://doi.org/10.1126/science.1153585>
- 1201 Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*, 34(18), 3094–  
1202 3100. <https://doi.org/10.1093/bioinformatics/bty191>
- 1203 Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform.  
1204 *Bioinformatics*, 25(14), 1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
- 1205 Li, H., & Durbin, R. (2024). Genome assembly in the telomere-to-telomere era. *Nature Reviews Genetics*,  
1206 25(9), 658–670. <https://doi.org/10.1038/s41576-024-00718-w>
- 1207 Li, J., Singh, U., Bhandary, P., Campbell, J., Arendsee, Z., Seetharam, A. S., & Wurtele, E. S. (2022). Foster  
1208 thy young: Enhanced prediction of orphan genes in assembled genomes. *Nucleic Acids Research*,  
1209 50(7), e37. <https://doi.org/10.1093/nar/gkab1238>
- 1210 Liang, P., Saqib, H. S. A., Zhang, X., Zhang, L., & Tang, H. (2018). Single-Base Resolution Map of  
1211 Evolutionary Constraints and Annotation of Conserved Elements across Major Grass Genomes.  
1212 *Genome Biology and Evolution*, 10(2), 473–488. <https://doi.org/10.1093/gbe/evy006>

- 1213 Lisch, D. (2013). How important are transposons for plant evolution? *Nature Reviews. Genetics*, 14(1),  
1214 49–61. <https://doi.org/10.1038/nrg3374>
- 1215 Llaca, V., & Messing, J. (1998). Amplicons of maize zein genes are conserved within genic but expanded  
1216 and constricted in intergenic regions. *The Plant Journal: For Cell and Molecular Biology*, 15(2),  
1217 211–220. <https://doi.org/10.1046/j.1365-313x.1998.00200.x>
- 1218 Lovell, J. T., Sreedasyam, A., Schranz, M. E., Wilson, M., Carlson, J. W., Harkess, A., Emms, D., Goodstein,  
1219 D. M., & Schmutz, J. (2022). GENESPACE tracks regions of interest and gene copy number  
1220 variation across multiple genomes. *eLife*, 11, e78526. <https://doi.org/10.7554/eLife.78526>
- 1221 Lu, Z., Marand, A. P., Ricci, W. A., Ethridge, C. L., Zhang, X., & Schmitz, R. J. (2019). The prevalence,  
1222 evolution and chromatin signatures of plant regulatory elements. *Nature Plants*, 5(12), 1250–  
1223 1259.
- 1224 Lynch, M., & Conery, J. S. (2000). The evolutionary fate and consequences of duplicate genes. *Science*,  
1225 290(5494), 1151–1155.
- 1226 Ma, J., & Bennetzen, J. L. (2004). Rapid recent growth and divergence of rice nuclear genomes.  
1227 *Proceedings of the National Academy of Sciences of the United States of America*, 101(34),  
1228 12404–12410. <https://doi.org/10.1073/pnas.0403715101>
- 1229 Ma, P.-F., Liu, Y.-L., Guo, C., Jin, G., Guo, Z.-H., Mao, L., Yang, Y.-Z., Niu, L.-Z., Wang, Y.-J., Clark, L. G.,  
1230 Kellogg, E. A., Xu, Z.-C., Ye, X.-Y., Liu, J.-X., Zhou, M.-Y., Luo, Y., Yang, Y., Soltis, D. E., Bennetzen,  
1231 J. L., ... Li, D.-Z. (2024). Genome assemblies of 11 bamboo species highlight diversification  
1232 induced by dynamic subgenome dominance. *Nature Genetics*, 56(4), 710–720.  
1233 <https://doi.org/10.1038/s41588-024-01683-0>
- 1234 Mallik, R., Wcisel, D. J., Near, T. J., Yoder, J. A., & Dornburg, A. (2023). *Investigating the impact of whole*  
1235 *genome duplication on transposable element evolution in ray-finned fishes* (p.  
1236 2023.12.22.572151). bioRxiv. <https://doi.org/10.1101/2023.12.22.572151>
- 1237 Mandáková, T., & Lysak, M. A. (2018). Post-polyploid diploidization and diversification through dysploid  
1238 changes. *Current Opinion in Plant Biology*, 42, 55–65. <https://doi.org/10.1016/j.pbi.2018.03.001>
- 1239 Marand, A. P., Chen, Z., Gallavotti, A., & Schmitz, R. J. (2021). A cis-regulatory atlas in maize at single-cell  
1240 resolution. *Cell*, 184(11), 3041-3055.e21. <https://doi.org/10.1016/j.cell.2021.04.014>
- 1241 Mason, A. S., & Wendel, J. F. (2020). Homoeologous Exchanges, Segmental Allopolyploidy, and Polyploid  
1242 Genome Evolution. *Frontiers in Genetics*, 11. <https://doi.org/10.3389/fgene.2020.01014>
- 1243 McClintock, B. (1941). The stability of broken ends of chromosomes in *Zea mays*. *Genetics*, 26(2), 234.
- 1244 McClintock, B. (1984). The significance of responses of the genome to challenge. *Science*, 226(4676),  
1245 792–801.
- 1246 McKain, M. R., Estep, M. C., Pasquet, R., Layton, D. J., Díaz, D. M. V., Zhong, J., Hodge, J. G., Malcomber,  
1247 S. T., Chipabika, G., Pallangyo, B., & Kellogg, E. A. (2018). *Ancestry of the two subgenomes of*  
1248 *maize* (p. 352351). bioRxiv. <https://doi.org/10.1101/352351>
- 1249 Morgante, M., Brunner, S., Pea, G., Fengler, K., Zuccolo, A., & Rafalski, A. (2005). Gene duplication and  
1250 exon shuffling by helitron-like transposons generate intraspecies diversity in maize. *Nature*  
1251 *Genetics*, 37(9), 997–1002. <https://doi.org/10.1038/ng1615>
- 1252 Morison, J. I., & Gifford, R. M. (1983). Stomatal sensitivity to carbon dioxide and humidity: A comparison  
1253 of two c(3) and two c(4) grass species. *Plant Physiology*, 71(4), 789–796.  
1254 <https://doi.org/10.1104/pp.71.4.789>
- 1255 Morrison, I. M. (1980). Changes in the lignin and hemicellulose concentrations of ten varieties of  
1256 temperate grasses with increasing maturity. *Grass and Forage Science*, 35(4), 287–293.  
1257 <https://doi.org/10.1111/j.1365-2494.1980.tb01525.x>
- 1258 Muiño, J. M., de Bruijn, S., Pajoro, A., Geuten, K., Vingron, M., Angenent, G. C., & Kaufmann, K. (2016).  
1259 Evolution of DNA-Binding Sites of a Floral Master Regulatory Transcription Factor. *Molecular*  
1260 *Biology and Evolution*, 33(1), 185–200. <https://doi.org/10.1093/molbev/msv210>

- 1261 Olson, A. J., & Ware, D. (2021). Ranked choice voting for representative transcripts with TRaCE.  
1262 *Bioinformatics*, 38(1), 261–264. <https://doi.org/10.1093/bioinformatics/btab542>
- 1263 Osborn, T. C., Chris Pires, J., Birchler, J. A., Auger, D. L., Jeffery Chen, Z., Lee, H.-S., Comai, L., Madlung,  
1264 A., Doerge, R. W., Colot, V., & Martienssen, R. A. (2003). Understanding mechanisms of novel  
1265 gene expression in polyploids. *Trends in Genetics*, 19(3), 141–147.  
1266 [https://doi.org/10.1016/S0168-9525\(03\)00015-5](https://doi.org/10.1016/S0168-9525(03)00015-5)
- 1267 Otto, S. P. (2007). The Evolutionary Consequences of Polyploidy. *Cell*, 131(3), 452–462.  
1268 <https://doi.org/10.1016/j.cell.2007.10.022>
- 1269 Ou, S., Su, W., Liao, Y., Chougule, K., Agda, J. R. A., Hellinga, A. J., Lugo, C. S. B., Elliott, T. A., Ware, D.,  
1270 Peterson, T., Jiang, N., Hirsch, C. N., & Hufford, M. B. (2019). Benchmarking transposable  
1271 element annotation methods for creation of a streamlined, comprehensive pipeline. *Genome*  
1272 *Biology*, 20(1), 275. <https://doi.org/10.1186/s13059-019-1905-y>
- 1273 Papon, N., Lasserre-Zuber, P., Rimbart, H., De Oliveira, R., Paux, E., & Choulet, F. (2023). All families of  
1274 transposable elements were active in the recent wheat genome evolution and polyploidy had no  
1275 impact on their activity. *The Plant Genome*, 16(3), e20347.
- 1276 Parisod, C., Holderegger, R., & Brochmann, C. (2010). Evolutionary consequences of autopolyploidy. *New*  
1277 *Phytologist*, 186(1), 5–17.
- 1278 Paterson, A. H., Chapman, B. A., Kissinger, J. C., Bowers, J. E., Feltus, F. A., & Estill, J. C. (2006). Many  
1279 gene and domain families have convergent fates following independent whole-genome  
1280 duplication events in Arabidopsis, Oryza, Saccharomyces and Tetraodon. *Trends in Genetics*,  
1281 22(11), 597–602.
- 1282 Phillips, A. R., Seetharam, A. S., Albert, P. S., AuBuchon-Elder, T., Birchler, J. A., Buckler, E. S., Gillespie, L.  
1283 J., Hufford, M. B., Llaca, V., Romay, M. C., Soreng, R. J., Kellogg, E. A., & Ross-Ibarra, J. (2023). A  
1284 happy accident: A novel turfgrass reference genome. *G3: Genes/Genomes/Genetics*, 13(6),  
1285 jkad073. <https://doi.org/10.1093/g3journal/jkad073>
- 1286 Pielou, E. C. (1966). The measurement of diversity in different types of biological collections. *Journal of*  
1287 *Theoretical Biology*, 13, 131–144. [https://doi.org/10.1016/0022-5193\(66\)90013-0](https://doi.org/10.1016/0022-5193(66)90013-0)
- 1288 Pikaard, C. S. (2001). Genomic change and gene silencing in polyploids. *TRENDS in Genetics*, 17(12), 675–  
1289 677.
- 1290 Poplin, R., Chang, P.-C., Alexander, D., Schwartz, S., Colthurst, T., Ku, A., Newburger, D., Dijamco, J.,  
1291 Nguyen, N., Afshar, P. T., & others. (2018). A universal SNP and small-indel variant caller using  
1292 deep neural networks. *Nature Biotechnology*, 36(10), 983–987.
- 1293 Pulido, M., & Casacuberta, J. M. (2023). Transposable element evolution in plant genome ecosystems.  
1294 *Current Opinion in Plant Biology*, 75, 102418. <https://doi.org/10.1016/j.pbi.2023.102418>
- 1295 Ramsey, J., & Schemske, D. W. (1998). PATHWAYS, MECHANISMS, AND RATES OF POLYPOID  
1296 FORMATION IN FLOWERING PLANTS. *Annual Review of Ecology, Evolution, and Systematics*,  
1297 29(Volume 29, 1998), 467–501. <https://doi.org/10.1146/annurev.ecolsys.29.1.467>
- 1298 Ramsey, J., & Schemske, D. W. (2002). Neopolyploidy in Flowering Plants. *Annual Review of Ecology,*  
1299 *Evolution, and Systematics*, 33(Volume 33, 2002), 589–639.  
1300 <https://doi.org/10.1146/annurev.ecolsys.33.010802.150437>
- 1301 Rawson, H. M., Begg, J. E., & Woodward, R. G. (1977). The effect of atmospheric humidity on  
1302 photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta*,  
1303 134(1), 5–10. <https://doi.org/10.1007/BF00390086>
- 1304 Rhoades, M. (1942). Preferential segregation in maize. *Genetics*, 27(4), 395.
- 1305 Rhoades, M., & Dempsey, E. (1972). On the mechanism of chromatin loss induced by the B chromosome  
1306 of maize. *Genetics*, 71(1), 73–96.
- 1307 Ricci, W. A., Lu, Z., Ji, L., Marand, A. P., Ethridge, C. L., Murphy, N. G., Noshay, J. M., Galli, M., Mejía-  
1308 Guerra, M. K., Colomé-Tatché, M., Johannes, F., Rowley, M. J., Corces, V. G., Zhai, J., Scanlon, M.

- 1309 J., Buckler, E. S., Gallavotti, A., Springer, N. M., Schmitz, R. J., & Zhang, X. (2019). Widespread  
1310 long-range cis-regulatory elements in the maize genome. *Nature Plants*, *5*(12), 1237–1249.  
1311 <https://doi.org/10.1038/s41477-019-0547-0>
- 1312 Roose, M. L., & Gottlieb, L. D. (1976). GENETIC AND BIOCHEMICAL CONSEQUENCES OF POLYPLOIDY IN  
1313 TRAGOPOGON1. *Evolution*, *30*(4), 818–830. [https://doi.org/10.1111/j.1558-](https://doi.org/10.1111/j.1558-5646.1976.tb00963.x)  
1314 [5646.1976.tb00963.x](https://doi.org/10.1111/j.1558-5646.1976.tb00963.x)
- 1315 Rosado, A., & Raikhel, N. V. (2010). Application of the gene dosage balance hypothesis to auxin-related  
1316 ribosomal mutants in Arabidopsis. *Plant Signaling & Behavior*, *5*(4), 450–452.  
1317 <https://doi.org/10.4161/psb.5.4.11341>
- 1318 Roulin, A., Auer, P. L., Libault, M., Schlueter, J., Farmer, A., May, G., Stacey, G., Doerge, R. W., & Jackson,  
1319 S. A. (2013). The fate of duplicated genes in a polyploid plant genome. *The Plant Journal*, *73*(1),  
1320 143–153. <https://doi.org/10.1111/tpj.12026>
- 1321 Salazar-Henao, J. E., Vélez-Bermúdez, I. C., & Schmidt, W. (2016). The regulation and plasticity of root  
1322 hair patterning and morphogenesis. *Development*, *143*(11), 1848–1858.  
1323 <https://doi.org/10.1242/dev.132845>
- 1324 Sanmiguel, P., & Bennetzen, J. L. (1998). Evidence that a Recent Increase in Maize Genome Size was  
1325 Caused by the Massive Amplification of Intergene Retrotransposons. *Annals of Botany*,  
1326 *82*(suppl\_1), 37–44. <https://doi.org/10.1006/anbo.1998.0746>
- 1327 SanMiguel, P., Gaut, B. S., Tikhonov, A., Nakajima, Y., & Bennetzen, J. L. (1998). The paleontology of  
1328 intergene retrotransposons of maize. *Nature Genetics*, *20*(1), 43–45.  
1329 <https://doi.org/10.1038/1695>
- 1330 Savadel, S. D., Hartwig, T., Turpin, Z. M., Vera, D. L., Lung, P.-Y., Sui, X., Blank, M., Frommer, W. B.,  
1331 Dennis, J. H., Zhang, J., & others. (2021). The native cistrome and sequence motif families of the  
1332 maize ear. *PLoS Genetics*, *17*(8), e1009689.
- 1333 Schnable, J. C., Springer, N. M., & Freeling, M. (2011). Differentiation of the maize subgenomes by  
1334 genome dominance and both ancient and ongoing gene loss. *Proceedings of the National  
1335 Academy of Sciences*, *108*(10), 4069–4074. <https://doi.org/10.1073/pnas.1101368108>
- 1336 Serapiglia, M. J., Gouker, F. E., Hart, J. F., Unda, F., Mansfield, S. D., Stipanovic, A. J., & Smart, L. B.  
1337 (2015). Ploidy Level Affects Important Biomass Traits of Novel Shrub Willow (*Salix*) Hybrids.  
1338 *BioEnergy Research*, *8*(1), 259–269. <https://doi.org/10.1007/s12155-014-9521-x>
- 1339 Shumate, A., & Salzberg, S. L. (2021). Liftoff: Accurate mapping of gene annotations. *Bioinformatics*,  
1340 *37*(12), 1639–1643. <https://doi.org/10.1093/bioinformatics/btaa1016>
- 1341 Sisodia, K. P. S. (1970). An interchange heterozygote in *Thelepogon elegans* roth ex roem et schult.  
1342 *Genetica*, *41*(1), 198–202. <https://doi.org/10.1007/BF00958905>
- 1343 Smit, A., Hubley, R., & Green, P. (2013). *RepeatMasker Open-4.0* <http://www.repeatmasker.org>.
- 1344 Soltis, D. E., Visger, C. J., Marchant, D. B., & Soltis, P. S. (2016). Polyploidy: Pitfalls and paths to a  
1345 paradigm. *American Journal of Botany*, *103*(7), 1146–1166. <https://doi.org/10.3732/ajb.1500501>
- 1346 Song, B., Buckler, E. S., Wang, H., Wu, Y., Rees, E., Kellogg, E. A., Gates, D. J., Khaiphob-Burch, M.,  
1347 Bradbury, P. J., Ross-Ibarra, J., Hufford, M. B., & Romay, M. C. (2021). Conserved noncoding  
1348 sequences provide insights into regulatory sequence and loss of gene expression in maize.  
1349 *Genome Research*, *31*(7), 1245–1257. <https://doi.org/10.1101/gr.266528.120>
- 1350 Song, B., Marco-Sola, S., Moreto, M., Johnson, L., Buckler, E. S., & Stitzer, M. C. (2022). AnchorWave:  
1351 Sensitive alignment of genomes with high sequence diversity, extensive structural  
1352 polymorphism, and whole-genome duplication. *Proceedings of the National Academy of  
1353 Sciences*, *119*(1), e2113075119. <https://doi.org/10.1073/pnas.2113075119>
- 1354 Spangler, R., Zaitchik, B., Russo, E., & Kellogg, E. (1999). Andropogoneae evolution and generic limits in  
1355 *Sorghum* (Poaceae) using *ndhF* sequences. *Systematic Botany*, 267–281.

- 1356 Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large  
1357 phylogenies. *Bioinformatics*, 30(9), 1312–1313.
- 1358 Stebbins, G. L. (1959). The Role of Hybridization in Evolution. *Proceedings of the American Philosophical*  
1359 *Society*, 103(2), 231–251.
- 1360 Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*.
- 1361 Stetter, M. G., Schmid, K., & Ludewig, U. (2015). Uncovering Genes and Ploidy Involved in the High  
1362 Diversity in Root Hair Density, Length and Response to Local Scarce Phosphate in *Arabidopsis*  
1363 *thaliana*. *PLOS ONE*, 10(3), e0120604. <https://doi.org/10.1371/journal.pone.0120604>
- 1364 Stiehler, F., Steinborn, M., Scholz, S., Dey, D., Weber, A. P. M., & Denton, A. K. (2021). Helixer: Cross-  
1365 species gene annotation of large eukaryotic genomes using deep learning. *Bioinformatics*,  
1366 36(22–23), 5291–5298. <https://doi.org/10.1093/bioinformatics/btaa1044>
- 1367 Stitzer, M. C., Khaipho-Burch, M. B., Hudson, A. I., Song, B., Valdez-Franco, J. A., Ramstein, G., Feschotte,  
1368 C., & Buckler, E. S. (2023). *Transposable element abundance subtly contributes to lower fitness in*  
1369 *maize* (p. 2023.09.18.557618). bioRxiv. <https://doi.org/10.1101/2023.09.18.557618>
- 1370 Sun, G., Wase, N., Shu, S., Jenkins, J., Zhou, B., Torres-Rodríguez, J. V., Chen, C., Sandor, L., Plott, C.,  
1371 Yoshinga, Y., Daum, C., Qi, P., Barry, K., Lipzen, A., Berry, L., Pedersen, C., Gottilla, T., Foltz, A.,  
1372 Yu, H., ... Schnable, J. C. (2022). Genome of *Paspalum vaginatum* and the role of trehalose  
1373 mediated autophagy in increasing maize biomass. *Nature Communications*, 13(1), 7731.  
1374 <https://doi.org/10.1038/s41467-022-35507-8>
- 1375 Tabatabaee, Y., Zhang, C., Warnow, T., & Mirarab, S. (2023). Phylogenomic branch length estimation  
1376 using quartets. *Bioinformatics*, 39(Supplement\_1), i185–i193.
- 1377 Tang, H., Zhang, X., Miao, C., Zhang, J., Ming, R., Schnable, J. C., Schnable, P. S., Lyons, E., & Lu, J. (2015).  
1378 ALLMAPS: Robust scaffold ordering based on multiple maps. *Genome Biology*, 16(1), 3.  
1379 <https://doi.org/10.1186/s13059-014-0573-1>
- 1380 Tayalé, A., & Parisod, C. (2013). Natural Pathways to Polyploidy in Plants and Consequences for Genome  
1381 Reorganization. *Cytogenetic and Genome Research*, 140(2–4), 79–96.  
1382 <https://doi.org/10.1159/000351318>
- 1383 Tremblay, B. J.-M. (2024). universalmotif: An R package for biological motif analysis. *Journal of Open*  
1384 *Source Software*, 9(100), 7012. <https://doi.org/10.21105/joss.07012>
- 1385 Tsukahara, S., Kobayashi, A., Kawabe, A., Mathieu, O., Miura, A., & Kakutani, T. (2009). Bursts of  
1386 retrotransposition reproduced in *Arabidopsis*. *Nature*, 461(7262), 423–426.  
1387 <https://doi.org/10.1038/nature08351>
- 1388 Tu, X., Mejía-Guerra, M. K., Valdes Franco, J. A., Tzeng, D., Chu, P.-Y., Shen, W., Wei, Y., Dai, X., Li, P.,  
1389 Buckler, E. S., & Zhong, S. (2020). Reconstructing the maize leaf regulatory network using ChIP-  
1390 seq data of 104 transcription factors. *Nature Communications*, 11(1), 5089.  
1391 <https://doi.org/10.1038/s41467-020-18832-8>
- 1392 Van de Peer, Y., Mizrachi, E., & Marchal, K. (2017). The evolutionary significance of polyploidy. *Nature*  
1393 *Reviews Genetics*, 18(7), 411–424. <https://doi.org/10.1038/nrg.2017.26>
- 1394 Vicent, C. M., Jääskeläinen, M. J., Kalendar, R., & Schulman, A. H. (2001). Active Retrotransposons Are a  
1395 Common Feature of Grass Genomes. *Plant Physiology*, 125(3), 1283–1292.  
1396 <https://doi.org/10.1104/pp.125.3.1283>
- 1397 Vitte, C., & Bennetzen, J. L. (2006). Analysis of retrotransposon structural diversity uncovers properties  
1398 and propensities in angiosperm genome evolution. *Proceedings of the National Academy of*  
1399 *Sciences*, 103(47), 17638–17643. <https://doi.org/10.1073/pnas.0605618103>
- 1400 Wang, H., & Bennetzen, J. L. (2012). Centromere retention and loss during the descent of maize from a  
1401 tetraploid ancestor. *Proceedings of the National Academy of Sciences*, 109(51), 21004–21009.
- 1402 Wang, Y., Tang, H., DeBarry, J. D., Tan, X., Li, J., Wang, X., Lee, T., Jin, H., Marler, B., Guo, H., Kissinger, J.  
1403 C., & Paterson, A. H. (2012). MCScanX: A toolkit for detection and evolutionary analysis of gene



- 1404 synteny and collinearity. *Nucleic Acids Research*, 40(7), e49.  
1405 <https://doi.org/10.1093/nar/gkr1293>
- 1406 Welker, C. A. D., McKain, M. R., Estep, M. C., Pasquet, R. S., Chipabika, G., Pallangyo, B., & Kellogg, E. A.  
1407 (2020). Phylogenomics enables biogeographic analysis and a new subtribal classification of  
1408 Andropogoneae (Poaceae—Panicoideae). *Journal of Systematics and Evolution*, 58(6), 1003–  
1409 1030. <https://doi.org/10.1111/jse.12691>
- 1410 Wendel, J., & Cronn, R. (2003). *Polyploidy and the evolutionary history of cotton*.
- 1411 Wendel, J. F. (2000). Genome evolution in polyploids. In J. J. Doyle & B. S. Gaut (Eds.), *Plant Molecular*  
1412 *Evolution* (pp. 225–249). Springer Netherlands. [https://doi.org/10.1007/978-94-011-4221-2\\_12](https://doi.org/10.1007/978-94-011-4221-2_12)
- 1413 Wendel, J. F. (2015). The wondrous cycles of polyploidy in plants. *American Journal of Botany*, 102(11),  
1414 1753–1756. <https://doi.org/10.3732/ajb.1500320>
- 1415 Wick, R. R., Judd, L. M., Gorrie, C. L., & Holt, K. E. (2017). Completing bacterial genome assemblies with  
1416 multiplex MinION sequencing. *Microbial Genomics*, 3(10), e000132.  
1417 <https://doi.org/10.1099/mgen.0.000132>
- 1418 Wicker, T., Gundlach, H., Spannagl, M., Uauy, C., Borrill, P., Ramírez-González, R. H., De Oliveira, R.,  
1419 Mayer, K. F. X., Paux, E., Choulet, F., & International Wheat Genome Sequencing Consortium.  
1420 (2018). Impact of transposable elements on genome structure and evolution in bread wheat.  
1421 *Genome Biology*, 19(1), 103. <https://doi.org/10.1186/s13059-018-1479-0>
- 1422 Wlodzimierz, P., Rabanal, F. A., Burns, R., Naish, M., Primetis, E., Scott, A., Mandáková, T., Gorringer, N.,  
1423 Tock, A. J., Holland, D., Fritsch, K., Habring, A., Lanz, C., Patel, C., Schlegel, T., Collenberg, M.,  
1424 Mielke, M., Nordborg, M., Roux, F., ... Henderson, I. R. (2023). Cycles of satellite and transposon  
1425 evolution in Arabidopsis centromeres. *Nature*, 618(7965), 557–565.  
1426 <https://doi.org/10.1038/s41586-023-06062-z>
- 1427 Xiong, Z., Gaeta, R. T., & Pires, J. C. (2011). Homoeologous shuffling and chromosome compensation  
1428 maintain genome balance in resynthesized allopolyploid Brassica napus. *Proceedings of the*  
1429 *National Academy of Sciences*, 108(19), 7908–7913. <https://doi.org/10.1073/pnas.1014138108>
- 1430 Zhang, C., Scornavacca, C., Molloy, E. K., & Mirarab, S. (2020). ASTRAL-Pro: Quartet-Based Species-Tree  
1431 Inference despite Paralogy. *Molecular Biology and Evolution*, 37(11), 3292–3307.  
1432 <https://doi.org/10.1093/molbev/msaa139>
- 1433 Zhao, M., Zhang, B., Lisch, D., & Ma, J. (2017). Patterns and Consequences of Subgenome Differentiation  
1434 Provide Insights into the Nature of Paleopolyploidy in Plants. *The Plant Cell*, 29(12), 2974–2994.  
1435 <https://doi.org/10.1105/tpc.17.00595>
- 1436 Zhou, Y., Li, S., Qian, Q., Zeng, D., Zhang, M., Guo, L., Liu, X., Zhang, B., Deng, L., Liu, X., Luo, G., Wang, X.,  
1437 & Li, J. (2009). BC10, a DUF266-containing and Golgi-located type II membrane protein, is  
1438 required for cell-wall biosynthesis in rice (*Oryza sativa* L.). *The Plant Journal*, 57(3), 446–462.  
1439 <https://doi.org/10.1111/j.1365-3113.2008.03703.x>
- 1440