RESEARCH ARTICLE

Subgenome Dominance in an Interspecific Hybrid, Synthetic Allopolyploid, and a 140-year-old Naturally Established Neo-allopolyploid Monkeyflower

Patrick P. Edger1,2,*, Ronald Smith3, Michael R. McKain4, Arielle M. Cooley5, Mario Vallejo-Marín6, Yaowu Yuan7, Adam J. Bewick8, Lexiang Ji9, Adrian E. Platts10, Megan J. Bowman11, Kevin L. Childs11,12, Jacob D. Washburn13, Robert J. Schmitz8, Gregory D. Smith3, J. Chris Pires13, and Joshua R. Puzey14,*,a

1Department of Horticulture, Michigan State University, East Lansing, MI
2Ecology, Evolutionary Biology and Behavior, Michigan State University, East Lansing, MI 48824
3Department of Applied Science, The College of William and Mary, Williamsburg, VA
4Donald Danforth Plant Science Center, St. Louis, MO
5Biology Department, Whitman College, Walla Walla, WA
6Biological and Environmental Sciences, University of Stirling, Stirling, UK
7Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT
8Department of Genetics, University of Georgia, Athens, GA
9Institute of Bioinformatics, University of Georgia, Athens, GA
10McGill Centre for Bioinformatics, McGill University, Montreal, QC, Canada H3A 0E9
11Department of Plant Biology, Michigan State University, East Lansing, MI 48824
12Center for Genomics Enabled Plant Science, Michigan State University, East Lansing, MI 48824
13Division of Biological Sciences, University of Missouri, Columbia, MO 65211
14Department of Biology, The College of William and Mary, Williamsburg, VA
* These authors contributed equally
aAuthor for correspondence: jrpuzey@wm.edu

Short Title: Emergence of subgenome dominance across time and ploidy

One-sentence summary: Subgenome differences in levels of transposon methylation mirror increases in subgenome specific expression bias observed over generations following hybridization and allopolyploidization in Mimulus.

ABSTRACT

Recent studies have shown that one of the parental subgenomes in ancient polyploids is generally more dominant - having retained more genes and being more highly expressed - a phenomenon termed subgenome dominance. The genomic features that determine how quickly and which subgenome dominates within a newly formed polyploid remain poorly understood. To investigate the rate of emergence of subgenome dominance, we examined gene expression, gene methylation, and transposable element (TE) methylation in a natural, less than 140 year old allopolyploid (Mimulus peregrinus), a resynthesized inter-species triploid hybrid (M. robertsii), a resynthesized allopolyploid (M. peregrinus), and progenitor species (M. guttatus and M. luteus). We show that subgenome expression dominance occurs instantly following the hybridization of divergent genomes and significantly increases over generations. Additionally, CHH methylation levels are reduced in regions...
neat genes and within TEs in the first-generation hybrid, intermediate in the resynthesized allopoly- 
oploid, and are repatterned differently between the dominant and recessive subgenomes in the natural allopoly- 
oploid. Subgenome differences in levels of TE methylation mirror the increase in expression bias observed over the generations following hybridization. These findings provide important insights into genomic and epigenomic shock that occurs following hybridization and polyploid events, and may also contribute to uncovering the mechanistic basis of heterosis and subgenome dominance.

INTRODUCTION

Whole genome duplications (WGD) have been an important recurrent process throughout the 
evolutionary history of eukaryotes (McLysaght et al., 2002; Dehal and Boore, 2005; Otto, 2007), including having contributed to the origin of novel traits and shifts in net diversification rates (Levin, 1983; Wright et al., 1998; Crow and Wagner, 2006; Chao et al., 2013; Edger et al., 2015; Tank et al., 2015). WGDs are especially widespread across flowering plants (Cui et al., 2006; Vanneste et al., 2014; Soltis and Soltis, 2016), with both deep WGD events (all extant angiosperms share at least two events (Jiao et al., 2011)) and a plethora of more recent events including those unique to our model system, Mimulus (Vallejo-Marín et al., 2015). Polyploids, species that have three or more complete sets of genomes, are grouped into two main categories: autopolyploids (WGD that occurred within a species) and allopolyploids (WGD coupled with an interspecific hybridization) (Ramsey and Schemske, 1998). Previous studies indicate that allopolyploids are more likely to persist and become ecologically established - a fact that has partially been attributed to heterosis due to transgressive gene expression and fixed heterozygosity (McLysaght et al., 2002; Crow and Wagner, 2006; Rapp et al., 2009; Barker et al., 2015). It is worth noting that this finding may be affected by the fact that it is often easier to identify allopolyploids than autopolyploids.

Newly formed allopolyploids face the unique challenge of organizing two genomes (i.e. subgenomes), each contributed by different parental species, that have independently evolved in separate contexts, but which now exist within a single nucleus (Comai, 2005). Homoploid hybridization and allopolyploidization may disrupt both genetic and epigenomic processes resulting in altered DNA methylation patterns (Shaked et al., 2001; Scheid et al., 2003; Salmon et al., 2005; Lukens et al., 2006; Chen, 2007; Song and Chen, 2015; Rigal et al., 2016), changes in gene expression (Adams et al., 2003; Adams and Wendel, 2005; Buggs et al., 2010; Chelaïfa et al., 2010; Coate et al., 2014; Renny-Byfield et al., 2015; Renny-Byfield et al., 2017) and transposable element (TE) reactivation (Dion-Côté et al., 2014) - commonly referred to as genomic shock (McClintock, 1984). These genome-wide changes are associated with novel phenotypic variation in newly formed
allopolyploids (Madlung et al., 2002; Gaeta et al., 2007), which likely contributed to the survival and ultimate success of polyploids (Kagale et al., 2014; Vanneste et al., 2014).

One observation that may be linked to the long-term success of allopolyploids is that homeologous genes (orthologous genes encoded on different parental subgenomes) are often expressed at non-equal levels, with genome-wide expression abundance patterns being highly skewed towards one of the subgenomes. Examples of plants with evidence for subgenome specific expression include maize (Zea mays, Schnable et al., 2011), Brassica (Cheng et al., 2016), cotton (Gossypium spp., Renny-Byfield et al., 2015), wheat (Li et al., 2014), Tragopogon (Buggs et al., 2010), Spartina (Chelaifa et al., 2010), and Arabidopsis thaliana (Wang et al., 2004). Additionally, it has been shown that the less expressed subgenome tends to be more highly fractionated (i.e. accumulate more deletions), a pattern thought to be due to relaxed selective constraints. Collectively, these phenomena are referred to as ‘subgenome dominance’ (Woodhouse et al., 2010). For example, in Brassica rapa, a three-way battle ensued following a whole genome triplication event that occurred over ten million years ago resulting in a single dominant subgenome emerging and two highly fractionated subgenomes (Tang et al., 2012). The most highly fractionated subgenome lost more than double the total number of genes than the least fractionated, dominant subgenome.

It remains largely unknown how one subgenome becomes more highly expressed, with respect to either whole genome patterns or specific genes. Another unanswered question is, on what time scale (i.e. how quickly) does subgenome dominance become established? Subgenome dominance in newly formed hybrids and allopolyploids could have substantial implications for our understanding of plant hybridization in both ecological and agricultural contexts. In addition, a mechanistic understanding of these phenomena is fundamental to better understanding the long-term evolutionary advantages of WGDs. One hint at a mechanism may be that gene expression can be impacted by the proximity to and methylation status of nearby TEs (Hollister and Gaut, 2009). Prompted by the finding that the density of methylated TEs is negatively correlated with gene expression magnitude, Freeling et al. (Freeling et al., 2012) hypothesized that the relationship between TE repression and the expression of neighboring genes might explain patterns of observed subgenome dominance. The degree of methylation repatterning and reestablishment genome-wide, specifically nearby genes, following hybridization and/or WGD is largely unknown. Here we tested this hypothesis by assessing (i) the overall rate that subgenome expression dominance is established following hybridization and WGD, (ii) genome-wide methylation repatterning following hybridization and WGD, and (iii) the influence of methylation repatterning on biased expression of parental subgenomes.
Research in most polyploid systems is hindered by at least one of two major difficulties: (1) lack of genomic resources for extant parental progenitors (if parents are known) or (2) the inability to confidently partition the polyploid genome to each of the parental subgenomes. Here we used the recently and recurrently formed natural allopolyploid, *Mimulus peregrinus*, to overcome these hurdles. *M. peregrinus* (6x) is derived from the hybridization of *M. luteus* (4x) and *M. guttatus* (2x), which produced a sterile triploid intermediate *M. x robertsii* (3x) that underwent a subsequent WGD to regain fertility (Vallejo-Marín, 2012; Vallejo-Marín et al., 2015) (Fig 1). Importantly, *M. luteus* (native to Chile) and *M. guttatus* (native to Western North America) only recently came into contact following a documented introduction into the UK in the early 1800’s (Vallejo-Marín et al., 2015). Thus, we have a narrow time window for the formation of *M. peregrinus*. Moreover, the natural allopolyploid (*M. peregrinus*) still exists with its introduced parents in the UK, which allows us to recreate hybrids and synthetic allopolyploids in the lab. Furthermore, the *M. guttatus* genome was recently published (Hellsten et al., 2013) and we complement this with a new genome assembly for *M. luteus*. *Mimulus luteus* is an allopolyploid formed from two unknown diploid progenitors, which may be long extinct due to the uncertain age of the polyploid event. These resources and the unique natural history of *M. peregrinus* have provided an unprecedented opportunity to properly investigate patterns of homeolog expression bias (measured by the ratio of expression of the homeologs) across subgenomes in a neo-allopolyploid and its relation to DNA methylation and TE density differences.

**RESULTS**

*Mimulus luteus* genome assembly

Here we present the draft genome of *M. luteus* with a total genome size estimate of 640–680 megabases (Mb) based on flow cytometry and kmer spectrum analysis (Vallejo-Marín, 2012). We sequenced the genome of an inbred line (EY7). The assembly contains 6,439 scaffolds spanning 410 Mb with an N50 of 283kb, representing roughly 60 percent of the genome, with gene content analyses supporting the recovery of nearly the entire gene space. A total of 46,855 protein-coding genes were annotated in *M. luteus* genome, which is nearly double the number of protein coding genes (26,718) previously annotated in the *M. guttatus* genome (430 Mb estimated; 300 Mb assembled) (Hellsten et al., 2013). This difference in gene content supports a tetraploid event unique to *M. luteus*, further supported by a 2:1 syntenic block ratio when compared to the *M. guttatus* genome, plus base chromosome number differences between these species (Vallejo-Marín, 2012a). The vast majority of BUSCO (Benchmarking Universal Single-Copy Orthologs) (Simão et al., 2015) groups, 931 of 956
(97.4 percent), were identified in the *M. luteus* genome assembly with 837 duplicates. The high number of identified duplicates supports both the tetraploid event and assembly completeness of the genic regions from both subgenomes. We mapped all mate pair reads back to the *M. luteus* assembly to screen for possible chimeric contigs or scaffolds between the two subgenomes (Lₐ and Lₕ). No instance of chimeric fusions between subgenomes was identified in the assembly (Supplemental Fig. 1 and 2), which was largely enabled by the overall sequence divergence of the two subgenomes (genome-wide average = 89.33% sequence similarity across genes) and leveraging overlapping paired-end sequences as single long reads. We reannotated the *M. guttatus* genome with identical methods used for *M. luteus*, reducing the total number to 25,465 protein coding genes. The reannotation of this genome permits us to make proper genomic and transcriptomic comparisons by removing artifacts that arise due to differences in genome annotation pipelines. A total of 319,944 and 451,448 TEs or TE fragments were annotated in the *M. guttatus* and *M. luteus* genomes, respectively. To annotate TEs, the TE exemplar library (see Methods) was used to identify TEs in the *M. guttatus* and *M. luteus* genomes using RepeatMasker. We combined these two genomes to represent *M. peregrinus.*

**History of WGD in Mimulus**

A shared ancient whole genome duplication was detected in both genomes, termed Mimulus-alpha, with a mean *Ks* of 0.92 and phylogenetically placed at the most recent common ancestor of Mimulus (Phrymaceae) and nearly all other Lamiales families (Fig. 1) (Supplemental Figs. 3, 4, and 5). Support was found at the base of the species tree for two possible WGD events: one prior to the diversification of all sampled Lamiales species and one after the divergence of Oleaceae. The PUG algorithm estimates the timing of WGD by identifying when coalescence of paralogs occurs in the context of a species tree. It is possible that the putative event placed after the divergence of Oleaceae is the result of artefactual gene tree reconciliation to the species tree. Another possibility is the event after the divergence of Oleaceae is the result of hybridization between the Oleaceae and the rest of Lamiales as suggested by Julca et al. (2017). Follow-up studies are needed to verify these findings with additional species sampling. A recent mean-date estimate for that phylogenetic node is 71 million years before present (Magallón et al., 2015), while other studies have obtained earlier and later date estimates (Tank et al., 2015; Wikström et al., 2015). Our taxon sampling did not include the earliest diverging lineage (family Plocospermataceae) in Lamiales (Refulio-Rodriguez and Olmstead, 2014; Magallón et al., 2015; Stull et al., 2015). The Mimulus-alpha event is shared by all other surveyed Lamiales families. A total of 757 unique shared duplications from Mimulus-alpha were identified in all surveyed taxa. Two additional duplication events detected across Lamiales were not
shared with Phrymaceae. The first detected event was shared by Orobanche (Orobanchaceae) and Striga (Oleaceae) and was supported by 1738 shared duplicate pairs. The second WGD event was shared by Olea and Fraxinus and supported by 3312 shared duplicate pairs.

*Mimulus luteus* experienced an additional whole genome duplication event not shared with *M. guttatus*. As a result, for every *M. guttatus* gene, *M. luteus* typically has two corresponding homeologs encoded on subgenomes (L_a and L_b). The mean per base divergence at neutral sites between the two *Mimulus luteus* subgenomes is ~0.11 Ks (synonymous substitutions per synonymous site). This level of divergence supports previous claim that this species is an allopolyploid (Mukherjee and Vickery, 1962) and by our previous genomic analysis, which suggested an allopolyploid origin for this taxon (Vallejo-Marín et al., 2015; Vallejo-Marín et al., 2016). Over fifty years ago, Mukherjee and Vickery (1962) hypothesized that *M. luteus* may be an allopolyploid formed by the hybridization of two distinct species with one more closely related to *M. guttatus* than the other species (Mukherjee and Vickery, 1962). We sought to test this hypothesis with phylogenetic analyses of syntenic orthologs between *M. guttatus* and from both *M. luteus* subgenomes. We determine that in the majority of cases (1853 of 2200 gene trees), homeologs encoded on subgenome A were more closely related to *M. guttatus* than it was to other subgenome supporting their hypothesis. However, it is important to note the *M. luteus* subgenomes are both quite distinct from *M. guttatus* at the sequence level (genome-wide average = 91.52% sequence similarity across genes). This suggests that the diploid progenitor species of either subgenome is likely not a close relative of *M. guttatus*, but rather a more distantly related species in the past. Thus, the newly formed allopolyploid *M. peregrinus* consists of three unique subgenomes; two from *M. luteus* (L_a and L_b) and one from *M. guttatus*.

**Investigating the establishment of subgenome dominance**

The goal of this study was to determine whether homeologs from a given subgenome are expressed at higher levels than homeologs from another subgenome. This goal guided our sampling strategy and analysis approach. Specifically, we sought to determine whether one homeolog was the dominantly expressed form over multiple tissue types (our goal is not to determine tissue-specific expression bias).

Towards this end, for each individual represented in this study (parents, hybrid, and natural allopolyploids), three separate RNA-seq datasets derived from three different tissues were generated. RNA-seq datasets for calyx, stem, and petals were generated for the F1 hybrid *M. x robertsii* (*M. guttatus* x *M. luteus*), the resynthesized allopolyploid *M. peregrinus*, and the naturally derived...
allopolyploid *M. peregrinus* and parental taxa, *M. luteus* and *M. guttatus*. For each tissue type we calculated the expression ratios of homeologs encoded on separate subgenomes. Thus our three biological replicates were the expression ratio of homeologs across three tissues. These data were used to measure homeolog-specific gene expression following the *M. luteus* WGD event as well as in a contemporary hybrid and neo-allopolyploids (*M. peregrinus*). All RNA samples were collected within a narrow time range to control for major diurnal rhythmic expression differences. In hybrids and allopolyploids, subgenome-specific (parental) single-nucleotide polymorphisms (SNPs) were identified and were used to measure homeolog specific gene expression. For the analysis of our RNA-seq data, we developed a likelihood ratio test (LRT) involving three nested hypotheses to identify cases of homeolog expression bias that do not involve tissue specific expression differences. The null hypothesis is that both homeologs are expressed at equal levels (ratio of homeolog-1 to homeolog-2 equals 1 for all three tissues). The first alternate hypothesis is that homeologs are expressed at different levels, but similar ratios, across all three tissue types. The second alternate hypothesis is that homeologs are expressed at different levels and at different ratios across all three tissues.

Using the expression data and the nested hypotheses we test for: (1) Expression bias and subgenome dominance following the *M. luteus* specific WGD, comparing $L_a$ and $L_b$ homeolog expression, in *M. luteus*, hybrid *M. guttatus x M. luteus*, and *M. peregrinus* (both natural and resynthesized allopolyploid) and (2) Expression bias and subgenome dominance of the *M. guttatus* or *M. luteus* homeologs in the hybrid and allopolyploid *M. peregrinus*. Importantly, the first comparison will allow us to understand long-term patterns of expression bias in *Mimulus* (the *M. luteus* WGD is a relatively ancient event) whereas the second comparison will test for expression bias and subgenome dominance in a newly formed hybrid and allopolyploid *Mimulus*.

**Expression bias of homeologs from *Mimulus luteus* specific WGD event**

We sought to compare expression of homeologs within *M. luteus* to each other, thereby addressing the question of whether $L_a$ homeologs or $L_b$ homeologs are more highly expressed within *M. luteus*, *M. x robertsii* (F1 hybrid), or *M. peregrinus* (both a resynthesized and natural allopolyploid). Using the likelihood ratio test mentioned above, we identified cases of homeolog expression bias that did not involve tissue-specific expression differences. In each case over 1100 homeolog pairs were tested; the test was only applied when both homeologs were expressed in all three tissues. For each homeolog pair, we quantified expression bias as $B = N^{-1} \sum_{j=1}^{N} \log_2 \left( \frac{\text{RPKM}_{b,j}}{\text{RPKM}_{a,j}} \right)$ where the subscripts $a$ and $b$ denote the two distinct subgenomes ($L_a$ and $L_b$), and $j$ is an index.
over the $N$ tissues. An expression bias of $\mathcal{B} = -2$ indicates a 4x expression bias towards the ‘a’ homeolog, while an expression bias of $\mathcal{B} = 3$ is 8x towards the ‘b’ homeolog. The histograms in Figure 2 summarize the measured expression bias of homeologs resulting from the $M. luteus$-specific WGD event in $M. luteus$, F1 hybrid, synthetic allopolyploid, and natural allopolyploid. For $M. luteus$ (top panel) the grey histogram shows the distribution of expression bias for all testable homeolog pairs indicating a slight average bias towards the $L_a$ subgenome ($\mathcal{B} = -0.09, N_{L_a} = 388 > 336 = N_{L_b}$) (Fig. 2). Using the likelihood ratio test developed in this paper, we found that over half of the homeolog pairs in $M. luteus$ ($N_{L_a} + N_{L_b} = 724$, about 53% of the total $N$) were biased towards one of the subgenomes with no tissue specific expression differences (hypothesis one, see Methods). Of these, a small majority of homeologs ($N_{L_a} = 388$, about 54% of biased homeologs), were dominantly expressed from the $L_a$ subgenome. In contrast to $M. luteus$, in the hybrid, synthetic allopolyploid, and natural allopolyploid, the $L_b$ subgenome is slightly dominant in either number or average (see panels two through four where about 52%, 51%, and 53% of biased homeologs are dominantly expressed from the $L_b$ subgenome and $\mathcal{B} = 0.05, 0.01, and 0.02$, respectively).

Expression bias of $M. guttatus$ and $M. luteus$ homeologs in the hybrid, resynthesized allopolyploid, and naturally occurring neo-allopolyploid Mimulus

To test for expression bias that arises instantaneously following the merger of two genomes, we compared homeologs in the hybrid, resynthesized allopolyploid and natural allopolyploid, which contain both a $M. guttatus$ and $M. luteus$ subgenome. We asked two questions. First, when considering $M. luteus$ weighted average of its homeologs compared to $M. guttatus$, do we see expression dominance towards either species (Fig. 3)? Second, when we consider the $M. luteus$ homeologs separately ($L_b$ or $L_a$) and compare these to their $M. guttatus$ homeolog ($G$), do we see expression dominance towards either species or a particular subgenome? The weighted average of expression of the two $M. luteus$ homeologs was calculated by dividing the sum of read count of the two $M. luteus$ homeologs by the sum of their individual gene lengths. The answer to the first question may indicate which parental species overall is more expressed while the second question will shed light whether one single subgenome is most dominant. For each homeolog pair, we quantified expression bias as $\mathcal{B} = N^{-1} \sum_{j=1}^{N} \log_2 \left( \frac{\text{RPKM}_j^{\ell}}{\text{RPKM}_j^g} \right)$, where the subscripts $\ell$ and $g$ denote the $M. luteus$ and $M. guttatus$ subgenome respectively, and $j$ is an index over the $N$ tissues. Considering the $M. luteus$ homeologs separately allows us to control for additive expression levels.
The histograms in Figure 3 summarize the measured expression bias in hybrid and allopolyploids, comparing the expression of the *M. guttatus* homeolog to the weighted average expression of its pair of *M. luteus* homeologs. This weighted average of expression is a proxy for the average expression activity of homeolog pairs from the *M. luteus* genome. On average, there is considerable bias towards the *M. luteus* subgenome with $\bar{B} = 0.57, 0.51$, and $1.05$ in the first generation hybrid, synthetic allopolyploid, and natural allopolyploid, respectively. Using the LRT (at significance level $\alpha = 0.01$) a total of 1893 (57%), 2072 (66%), and 2096 (70%) homeolog pairs were found to be significantly biased. Of these biased pairs, the *M. luteus* homeolog was dominant in the vast majority of cases ($N_e = 1330 (70\%), 1397 (67\%), 1573 (75\%)$, respectively).

The histograms in Figure 4 summarize the measured expression bias in hybrid and allopolyploids, comparing the expression of the *M. guttatus* homeolog to expression of each of its *M. luteus* homeologs separately. That is, for each *M. guttatus* gene (G), there are two *M. luteus* homeologs, $L_a$ and $L_b$. Figure 4 includes the comparisons: G vs $L_a$, and G vs. $L_b$. In other words, each G is used in 2 different comparisons. When considering the *M. luteus* homeologs separately, expression is still considerably biased on average towards *M. luteus* with $\bar{B} = 0.51, 0.48$, and $1.00$, in the first generation hybrid, resynthesized allopolyploid, and natural allopolyploid, respectively (Fig. 4). Next, using the LRT, across all comparisons, 55%, 64%, and 69% homeologs were significantly biased. Of these biased homeolog pairs, the *M. luteus* homeolog was the dominantly expressed homeolog in 69%, 65%, and 74% of the comparisons (Fig. 4). Additionally, among biased homeologs, the average bias towards the *M. luteus* subgenome is greater than the average bias towards the *M. guttatus* subgenome \(\left(\mid \bar{B}_e \mid > \mid \bar{B}_g \mid \right)\) in all cases (Fig. 4). It is also worth noting that the degree of bias (as measured by the fraction of biased homeologs and $B$ of biased homeologs) increased from the first generation hybrid, to the resynthesized allopolyploid, and to the natural allopolyploid.

**Expression bias in three separate hybrid lineages**

While it is clear that the *M. luteus* homologs are dominantly expressed in the hybrid and allopolyploid lineages, we sought to determine whether the same homeologs were repeatedly biased across independent hybrid and allopolyploids. A Venn diagram reveals that homeologs biased in one individual are far more likely to be biased in the other two lineages than would be expected by random chance (Fig. 5). Moreover, measured levels of individual homeolog expression bias are correlated across all three lineages (Fig. 5). Interestingly, levels of expression bias $B$ in the first
generation hybrid and resynthesized allopolyploid are much more correlated with each other ($r^2 = 0.70$) than either sample is with the natural allopolyploid ($r^2 = 0.35$ and 0.33, Fig. 5).

**Transposon density linked to gene expression**

One possibility we considered was whether proximal transposon (TE) loads were related to homeolog expression bias. In order to test this, it was necessary to annotate TEs in *M. guttatus* and *M. luteus* genome assemblies. Using a homology and structured based annotation, as well as de novo annotation, we identified the transposons in the *M. guttatus* and *M. luteus* genomes. Our survey revealed that 50% of the *M. guttatus* genome assembly is composed of TE sequences that are classified into 863 families. We have compiled a TE exemplar library with 1439 sequences representing the TE composition of the genome. After annotating TEs in 10-kb windows (10 kb upstream and downstream of genes as well as within genic region), we calculated the total number of TEs and the number of TE bases. On average, *M. luteus* homeologs and *M. guttatus* homeologs have TE densities of 0.31 and 0.34 (fraction of bases that occur within a transposon), respectively. In the parents, hybrid, and allopolyploid individuals, this measure of proximal TE density is negatively correlated with gene expression (Fig. 6 and Supplemental Fig. 6 and 7).

**Altered DNA methylation patterns in parents, hybrid, and allopolyploids**

Building on the finding of expression bias in the hybrid and neo-allopolyploids, we asked whether DNA methylation changes mirror the observed expression bias. First, whole genome bisulfite sequencing (WGBS) was used to determine the methylation status and patterns of methylation change in hybrid and neo-allopolyploid lineages as well as in each parent at CHH (where H = C, A, T), CHG, and CG sites. Next, we tested the hypothesis that changes in TE methylation between parents and hybrid or allopolyploids may explain patterns of subgenome dominance. Methylation patterns in TE and genes and their upstream and downstream regions were compared. CG and CHG methylation patterns in genes and TE are unchanged in the hybrid and allopolyploid lineages (Fig. 7). CHG methylation levels are marginally lower in upstream and downstream regions of genes in the hybrid and slightly higher in the synthetic and natural allopolyploid. CHH methylation levels are decreased significantly in upstream and downstream genic regions in the first generation hybrid, decreased slightly in the resynthesized allopolyploid, and returned to parental levels in the natural allopolyploid. Similar to findings in genes, transposon bodies and up- and downstream regions of transposons are depleted in CHH methylation in the hybrid and synthetic allopolyploid (Fig. 7 and Supplemental Fig. 8). Transposon CHH methylation levels are lowest in the first
generation hybrid and remained at a reduced level in the resynthesized allopolyploid compared to parental levels. In the natural allopolyploid, CHH methylation of TEs across the *M. guttatus* subgenome returned to near parental levels while *M. luteus* subgenome TE methylation remained lower compared to parental levels. Methylation repatterning across the subgenomes closely reflects the pattern of homeolog expression bias observed between the two subgenomes in the hybrid and allopolyploids.

**DISCUSSION**

Investigating the aftermath of WGDs across both deep and recent time scales provides clearer insight into the collective evolutionary processes that occur in a polyploid nucleus (Mayfield-Jones et al., 2013), including the emergence and establishment of subgenome dominance. Subgenome dominance has largely been investigated in ancient polyploids, including Arabidopsis (Thomas et al., 2006), maize (Schnable et al., 2011) and *Brassica* (Cheng et al., 2016), which revealed the presence of a dominant subgenome with significantly greater gene content and which contributes more to the global transcriptome than the other subgenome(s).

Gene expression bias towards one of the subgenomes has also been observed in more recent allopolyploids including those formed as a product of domestication over the past ten thousand years (e.g. wheat (Li et al., 2014) and within recently formed natural allopolyploids, namely *Tragopogon mirus* (Buggs et al., 2010). Due to the recent time scale of these WGD events, gene fractionation bias towards the recessive subgenome is not observed. It remains largely unknown how quickly subgenome dominance is established following an allopolyploid event. Subgenome dominance can also manifest itself in ways other than homeolog expression bias. For instance, in *Nicotiana* repeat abundances and rDNA levels exhibit subgenome specific changes (Kovarik et al., 2008; Renny-Byfield et al., 2011), and in *Brassica* expression bias of rRNA genes from a single parent in a hybrid or allopolyploid has been observed (Chen and Pikaard, 1997). In the future, exploring these questions in *Mimulus* may lead to a more comprehensive understanding of subgenome bias.

Here we report that subgenome dominance becomes established in the first generation hybrid of two *Mimulus* species. Our analyses show that homeologs from *M. luteus*, compared to *M. guttatus*, are significantly more expressed in the interspecific F1 hybrid and that this expression bias increases over subsequent generations, with the greatest bias observed in the natural (≈ 140 year old) neo-allopolyploid *M. peregrinus*. Using the LRT we determined that the number of biased homeolog pairs also increases with additional generations.
Genome-wide methylation analyses uncovered that CHH methylation levels are greatly reduced in the F1 hybrid. The greatest changes in CHH methylation are observed near gene bodies, near TE bodies, and within TE bodies. The methylation status of many of these CHH sites are regained in the second-generation resynthesized allopolyploid; indicating the onset of repatterning of DNA methylation. The methylation status of CHH sites near genes returned to parental levels across both subgenomes in the natural allopolyploid. Similarly, the CHH methylation status of TEs across *M. guttatus* subgenome returned to near parental levels in the natural allopolyploid. However, this pattern for CHH methylation is not observed across the dominant *M. luteus* subgenomes in the natural allopolyploid, with methylation levels within and near TEs remaining noticeably below parental levels. The methylation of CHG sites near TE and gene bodies were also impacted upon hybridization, but to a lesser degree than CHH methylation, and quickly returned to either at or above parental levels in the resynthesized allopolyploid. It is important to note that although *M. luteus* has 84% percent more genes than *M. guttatus*, it only has 41% percent more TEs. This means that the *M. luteus* genome has evolved to have fewer TEs at a genome-wide level. These observations, a dominantly expressed subgenome with lower TE abundance and lower CHH methylation levels near genes compared to the recessive subgenome, support the predictions made by Freeling et al. (Freeling et al., 2012) to explain subgenome dominance. However, given these data alone, we are unable at this time to distinguish between causation and correlation.

Our analyses confirm that the density of TEs negatively impacts the expression of nearby genes in *Mimulus*, similar to observations made in Arabidopsis (Hollister and Gaut, 2009). Furthermore, here we show that the repatterning of TE methylation levels is different for the two subgenomes in *M. peregrinus*, mirroring the expression bias observed over the generations following the hybridization. Our results suggest that subgenome dominance may be at least partially due to subgenome-specific differences in the epigenetic silencing of TEs, which was established long ago in the ancestors of the diploid progenitors. The strong correlation between homeolog expression bias (*B*) in the F1 hybrid, resynthesized allopolyploid, and independently established natural allopolyploid indicate that the observed subgenome expression dominance is biologically real and likely heritable. The fact that homeolog expression bias (*B*) in *Mimulus* hybrids mirrors methylation repatterning and subgenome specific TE densities supports the original hypothesis for the mechanistic basis of subgenome dominance.

The observed methylation differences between homeologs present on the different subgenomes may
represent early earmarks for the ultimate loss (i.e. fractionation) of a duplicate gene copy.

Fractionation is the loss of genes, regulatory elements, or other genomic features from a subgenome over time. Recessive subgenomes in ancient polyploids may be more highly fractionated and contribute less to the overall transcriptome compared to the dominant subgenome(s) (Tang et al., 2012).

Although duplicate genes on either subgenome are not physically lost yet in *M. peregrinus*, many homeologs on the recessive subgenome are already functionally absent (low to no expression). Due to selection acting on maintaining proper stoichiometry in dosage-sensitive macromolecular complexes and gene-interaction networks (Birchler et al., 2007; Edger and Pires, 2009), stoichiometric balance is likely best maintained by retaining the more highly expressed copy of interacting genes. One of the biggest opportunities arising from any gene duplication is the possibility of sub- or neo-functionalization. The finding of strong and immediate homeolog expression bias in a hybrid and neo-allopolyploid may have important implications for our understanding of these processes.

Additionally, our analyses revealed that the two subgenomes in *M. luteus* are each dominant over the *M. guttatus* subgenome in the F1 hybrid and allopolyploid *M. peregrinus*. This observed pattern of dominance between the three subgenomes in *Mimulus* is opposite of what was reported for *Brassica rapa* (Tang et al., 2012), which similarly underwent a whole genome triplication. Tang et al. (2012) hypothesized that the subgenome which joined the “battle” last, in a two-step process towards hexaploidy, emerged as the dominantly expressed and gene rich subgenome. In resynthesized and natural *M. peregrinus*, the most dominantly expressed subgenomes joined the “battle” first in *M. luteus* which are both dominant over the *M. guttatus* subgenome. This suggests that the dominant subgenome(s) in higher polyploids (e.g. hexa-, octo-, and deca-) is likely not determined by the order by which subgenomes are merged into a single nucleus. Instead, our results collectively suggest that subgenome dominance is at least partially due to subgenome-specific epigenetic differences.

In conclusion, there appears to be clear tradeoff between the benefits of epigenetic silencing of TEs (this inhibits their proliferation across the genome) and the effects of TE methylation on neighboring gene expression (Hollister and Gaut, 2009). Our results support the idea that subgenome dominance may be the result of lineage-specific genomic evolution shaping TE densities and methylation levels. In addition, subgenome expression dominance should not be unique to interspecific hybrids, but should also occur in intraspecies crosses between lines with different TE loads. These results have major implications to a number of research fields ranging from ecological studies to crop breeding efforts.
METHODS

Genome assembly and annotation

Genome assembly of M. luteus was performed using the ALLPATHS-LG assembler v45395 (Gnerre et al., 2011) with approximately 33x Illumina paired-end (2x100 bp) reads and a TruSeq mate-pair (5-kb insert) library. Additional gap closing was undertaken using GapCloser (v. 1.12, BGI). The assembly totaled 409 Mb in 6,349 scaffolds with a scaffold N50/L50 of 283/439 kb, a contig N50 of 52 kb and 96 percent of bases called (IUPAC ambiguous bases converted to N). This is 60 percent of the total genome size (680 Mb) anticipated by the assembler from the Kmer depth distribution.

Genome completeness in terms of gene content was assessed using BUSCO (Simão et al., 2015) with default settings and a set of universal single-copy orthologs. A read coverage depth analysis was also run to verify that homeologous regions in the M. luteus genome were not collapsed during the assembly. TEs were annotated using a combination of sequence and structure homology as well as de novo approaches (see TE annotation methods).

The MAKER genome annotation pipeline was used to annotate the M. luteus and M. guttatus genome assemblies using SNAP, AUGUSTUS and Fgenesh gene prediction programs (Salamov and Solovyev, 2000; Stanke and Waack, 2003; Korf, 2004; Cantarel et al., 2008). Species-specific transcript assemblies as well as TAIR Arabidopsis thaliana and plant-specific SwissProt protein sequences were aligned to the repeat-masked genome assemblies and used as evidence to aid the gene prediction programs (Berardini et al., 2015). Gene predictions with transcript, protein or protein domain support were retained as final high-quality gene predictions (Campbell et al., 2014). The assembled genome sequence and annotation files (GFFs), including gene models, are deposited on CoGe (https://genomevolution.org/coge; Genome IDs 22656 and 22665) and Dryad (DOI: doi:10.5061/dryad.d4v0).

Whole genome alignments and phylogenetic analyses

Pairwise genomic alignments between the M. luteus and M. guttatus genomes were made using SynMap (Lyons et al., 2008) and QUOTA-ALIGN (Tang et al., 2011), and then filtered to identify syntenic orthologs between the two genomes and retained homeologs in M. luteus. Data for various members of the order Lamiales were downloaded from NCBI-SRA and combined with newly generated transcriptome data for M. peregrinus and genomic data from M. luteus, M. guttatus (v.1.2) (Hellsten et al., 2013), and Solanum lycopersicum (ITAG2.3) (Consortium and others, 2012) for phylogenetic analysis of retained duplicates.

Transcriptomes were assembled using Trinity v.2.1.1 (Haas et al., 2013). Reads were filtered for quality and length with adapter trimming (ILLUMINACLIP:TruSeq2-PE.fa:2:30:10), a sliding
window of 10 bases with an average phred score of 20 (SLIDINGWINDOW:10:20), and a minimum length of 40 base pairs (MINLEN:40). Reads were normalized using Trinity’s in silico read normalization option with default parameters and a max coverage of 50 and assembled as paired-end with no assumed directionality. Assemblies were FPKM (per kilobase of exon per million fragments mapped) filtered by aligning reads to assembled contigs using bowtie v.0.12.7 (Langmead and Salzberg, 2012), abundance was estimated using RSEM v.1.2.17 (Li and Dewey, 2011) and the “align_and_estimate_abundance.pl” script available with Trinity. Isoforms with a minimum of 1% of total mapped fragments to a component were kept as well-supported assemblies.

Transcripts were translated using the same method as McKain et al. (2016). For translation, the coding sequence (CDS) from *Mimulus guttatus* was used as a reference for the RefTrans pipeline (https://github.com/mrmckain/RefTrans) and GeneWise v.2.2.0 (Birney et al., 2004). Assembled contigs were BLASTed (BLASTX) against amino acid sequences from a reference dataset with a minimum e-value cutoff of 1e-10. Reference sequences were identified by filtering all BLASTX hits with a bidirectional overlap of 85% for each contig-reference pair.

Orthogroups were identified using OrthoFinder v.0.4 (Emms and Kelly, 2015) from translated transcriptome and full genome data sets with default parameters. Sequences were sorted into individual orthogroups, and amino acids were aligned using PASTA v.1.6.3 (Mirarab et al., 2014) under default parameters. CDS was overlaid to amino acid alignments using PAL2NAL v.14 (Suyama et al., 2006). Codon alignments from CDS were used to reconstruct gene trees using RAxML v.7.3.0 (Stamatakis, 2006) under a GTR+gamma model and 500 bootstrap replicates.

Single-copy gene trees were identified using the clone_reducer method outlined in (Estep et al., 2014) and (McKain et al., 2016). In short, for each gene family tree, clades with bootstrap values of 50 or greater that consisted of only a single taxon were reduced to a single, longest representative sequence. A total of 96 single copy orthogroups were identified. Reduced orthogroup phylogenies were then estimated in RAxML under a GTR+gamma model with 500 bootstrap replicates. A coalescence-based tree was estimated using Astral v.4.10.2 (Mirarab and Warnow, 2015) with 100 bootstrap replicates using the 96 single copy orthogroup trees and their associated bootstrap trees. A concatenation-based tree was estimated by first concatenating alignments for all 96 orthogroups, and then estimating the phylogeny using RAxML under a GTR+gamma model with 500 bootstrap replicates.

Both the concatenation and coalescence species trees were used as guide trees to estimate the phylogenetic placement of putative whole genome duplication events with the PUG software (https://github.com/mrmckain/PUG; (McKain et al., 2016)). PUG was used to identify all putative paralog pairs for all sampled taxa across non-single copy gene families. A total of 1,731,351 putative
paralogs were estimated from 10,888 gene trees. For each putative paralog pair, PUG identifies the node of coalescence in a gene tree and queries the subtree against the species tree. PUG then verifies that the taxa present in the sister lineage to the minimal species subtree are also present in the lineage sister to the paralog subtree. If this is found to be true, the paralog subtree is counted as a gene duplication event. The bootstrap value of the paralog coalescence node is recorded and all coalescence subtrees are filtered for minimal bootstrap values of 50 and 80. All data presented in this manuscript are for nodes with a bootstrap value of at least 80.

**Mimulus individuals used in this study**

Two outbred individuals of *M. guttatus* and *M. luteus* s.l. from two populations in the United Kingdom (DBL and COL, respectively), derived from manual cross-fertilization of field-collected plants, were hybridized to produce triploid seed as described in Vallejo-Marin et al. (2016). Individuals from these inbred lines were hybridized to produce triploids as described in Vallejo-Marin et al. (Vallejo-Marin et al., 2016). Triploid seeds from a single hybrid cross (H003b; product of crossing accessions CG-1-1 and CS-4-3) were treated in 2013 with an aqueous solution of 0.1% colchicine to induce somatic polyploidization. Seeds were then planted, grown to flower, and screened with flow cytometry. A single mixoploid individual (3x and 6x nuclei; SYN-1) producing fertile pollen was self-pollinated to generate viable seeds. These seeds were then germinated and screened using flow cytometry to confirm that they were stable hexaploids (13-SYN-seed). For details on localities and taxonomy of the accessions used in the hybridization experiment see Vallejo-Marin et al. (2016).

**RNA sequencing and quantification of gene expression**

RNA-seq libraries were constructed using the TruSeq RNA kit (Illumina; San Diego, CA) from total RNA isolated from calyx, stem, and petals and then sequenced with single-end 100bp reads on an Illumina HiSeq-2000 at the University of Missouri Sequencing Core for three tissue types for both *M. guttatus* and *M. luteus*. Illumina reads were quality filtered using NextGENe v2.3.3.1 (SoftGenetics; State College, PA), removing adapter sequences, reads with a median quality score of less than 22, trimmed reads at positions that had three consecutive bases with a quality score of less than 20, and any trimmed reads with a total length less than 40 bp. This resulted in 87.9 percent of the reads passing the quality-score filter. Expression levels, in FPKM (fragments per kilobase per million reads), were determined for all genes in the *M. guttatus* and *M. luteus* genomes. Quality-filtered reads for each library were aligned to the respective genomes using NextGENe v2.3.3.1 and counting only uniquely mapped reads, using parameters (A. matching Requirement: >40 Bases and >99 percent, B. Allow Ambiguous Mapping: FALSE, and C. Rigorous Alignment: TRUE) resulting in the alignment
of over 189.6 million reads to the *M. guttatus* and *M. luteus* genomes. The raw read counts and normalized RPKM values for each gene and every library used in this study are also deposited on Dryad (DOI: doi:10.5061/dryad.d4vr0 ). Using these data, we compared expression of each individual homeolog (*L*b, *L*a or G) to each other. We also compared the expression of *M. luteus* to *M. guttatus* by comparing the weighted average of *M. luteus*’ two homeologs to *M. guttatus*. To do this, the expression of the two *M. luteus* homeologs was calculated by dividing the sum of read count of the two *M. luteus* homeologs by the sum of their individual gene lengths (weighted average). The Venn diagram of biased genes (Fig. 5A) was drawn using eulerAPE (Micallef and Rodgers, 2014).

**Whole genome methylation sequencing and analysis**

MethylC-seq libraries were prepared according to the following protocol (Urich et al., 2015), which involves sodium bisulfite treatment and the deamination of unmethylated cytosines to uracil, while methylated cytosines remain the same. Libraries were Illumina HiSeq 2500 platform with 150-bp reads at the University of Georgia. Cutadapt v1.9 (Martin, 2011) was used to trim adapter sequences, and then Bowtie 1.1.0 (Langmead and Salzberg, 2012) aligned reads to both a converted forward strand (cytosines to thymines) and converted reverse strand (guanines to adenines) reference genomes as previously described in (Schmitz et al., 2013). Residue substitution of the reference genome adjusts for sodium bisulfite conversion and strand of the reads. Only uniquely aligned reads were retained. Mitochondrial sequence of *M. guttatus* (which is fully unmethylated) was used as control to calculate the sodium bisulfite reaction non-conversion rate of unmodified cytosines. For metaplots, both upstream and downstream regions were divided into 20 bins each of 50bp in length for a total 1 kb in each direction. Gene and TE bodies were separated every 5 percent, for a total of 20 bins. Weighted methylation levels were computed for each bin as described previously (Schultz et al., 2012). These data are deposited on GEO (GSE95799).

**LRT to detect homeolog expression bias**

We defined homeolog expression bias (*B*) as

\[
B = \frac{1}{N} \left( \sum_{j=1}^{N} \log_{2} \frac{\text{RPKM}_B}{\text{RPKM}_A} \right)
\]

where *A* and *B* represent *M. luteus* and *M. guttatus*. In this formula, the observed expression levels of the homeologs are normalized by gene length and the total number of mapped reads and *j* is an index over the *N* tissues. This measure of homeolog expression bias can be computed for any homeolog pair.
with non-zero read counts (testable homeolog pairs).

For the analysis of our RNA-seq data, we developed a likelihood ratio test involving three nested hypotheses to identify cases of homeolog expression bias that do not involve tissue specific expression differences. The null hypothesis ($H_0$) is that homeologs are expressed at equal levels (ratio of homeolog-1 to homeolog-2 equals 1 for all three tissues). The first alternative hypothesis ($H_1$) is that homeologs are expressed at different levels, but similar ratios, across all three tissue types. The second alternative hypothesis ($H_2$) is that homeologs are expressed at different levels and at different ratios across all three tissues.

A brief description of our likelihood ratio test for homeolog expression bias that does not involve tissue specific expression differences follows (Smith et al., 2017). Denote the true but unknown expression levels (per kilobase of coding DNA sequence) of homeologs $A$ and $B$ as $\Lambda^A_j$ and $\Lambda^B_j$. The expected number of reads in tissue $j$ ($\lambda^A_j$ and $\lambda^B_j$) are

$$\lambda^A_j = \Lambda^A_j k^A d$$
$$\lambda^B_j = \Lambda^B_j k^B d$$

where $k^A$ and $k^B$ and are the transcript lengths of homeologs $A$ and $B$, respectively, and $d$ is the total number of reads generated. Define $\alpha_j = \frac{\Lambda^B_j}{\Lambda^A_j}$ to be the ‘expression scaling factor’ of $A$ compared to $B$ in tissue $j$, and $K = \frac{k^B}{k^A}$ to be the ratio of the homeologs’ transcript lengths. In that case, the expected number of reads, $\lambda^A_j$ and $\lambda^B_j$, are related by

$$\lambda^B_j = \alpha_j K \lambda^A_j$$

where $K = \frac{k^B}{k^A}$ is a known quantity.

Because we are working with single replicates (one observation for each homeolog pair in each tissue), we model the observed read counts ($X^A_j$ and $X^B_j$ ) as Poisson distributed random variables with parameters $\lambda^A_j$ and $\lambda^B_j$ respectively. The parameter sets associated with the three nested hypotheses for our likelihood ratio test are as follows:

$\Theta_0: \{\lambda_1, \ldots, \lambda_N, \alpha_1, \ldots, \alpha_N : \lambda_j > 0 \text{ and } \alpha_j = 1 \text{ where } j = 1, \ldots, N\}$

$\Theta_1: \{\lambda_1, \ldots, \lambda_N, \alpha_1, \ldots, \alpha_N : \lambda_j > 0 \text{ and } \alpha_j = \alpha > 0 \text{ where } j = 1, \ldots, N\}$
\[ \Theta_2: \{ \lambda_1, ..., \lambda_N, \alpha_1, ..., \alpha_N : \lambda_j > 0 \text{ and } \alpha_j > 0 \text{ where } j = 1, ..., N \} \]

where \( \Theta_0 \subset \Theta_1 \subset \Theta_2 \). The parameter set \( \Theta_0 \) corresponds to the null hypothesis (\( H_0 \)) that, after accounting for differences in transcript length, there are no tissue- or subgenome-specific differences in expression levels. The parameter set \( \Theta_1 \) corresponds the first alternative hypothesis (\( H_1 \)) that there are subgenome- but not tissue-specific differences in expression levels. The parameter set \( \Theta_2 \) corresponds to the second alternative hypothesis (\( H_2 \)) that there are tissue-specific differences in expression levels.

Assuming the probability models described above, we analytically derived the likelihood functions for \( H_0, H_1 \) and \( H_2 \). Using the observed counts for each homeolog pair \( X^A_j \) and \( X^B_j \), maximum likelihood estimation of the free parameters was performed using MATLAB’s built-in nonlinear system solver (fsolve) for \( H_1 \), while \( H_0 \) and \( H_2 \), while \( H_0 \) and \( H_2 \) admit analytic solutions.

Subsequently, two likelihood ratio tests were performed (using significance levels of 0.01). The first test was to determine whether we could reject \( H_0 \) in favor of \( H_1 \). If so, a second test determined whether we could reject \( H_1 \) in favor of \( H_2 \). Rejecting \( H_0 \), but not rejecting \( H_1 \), is interpreted as statistically significant homeolog expression bias that does not involve tissue specific expression differences. The Benjamini-Hochberg correction (Benjamini and Hochberg, 1995) was made to correct for multiple testing error for the first test, but not the second, since we were asking whether we were unable to reject \( H_1 \) in favor of \( H_2 \).

Methods for transposon annotation

**Autonomous cut-and-paste TEs**

There are five superfamilies of cut-and-paste DNA TEs in angiosperm genomes [i.e., Tc1/mariner, PIF/Harbinger, Mutator-like elements (MULEs), hAT, and CACTA] (Yuan and Wessler, 2011). They can be readily distinguished by the size of their target-site duplications (TSDs) and sequence similarity of the encoded transposase (TPase). The DDE/D domain alignment profile for each of the five superfamilies (obtained from (Yuan and Wessler, 2011)) was used as query to search against the *Mimulus guttatus* genome assembly by TBLASTN (Altschul et al., 1997), as implemented in the TARGeT pipeline (Han et al., 2009). TARGeT outputs the DNA sequences with 10 kb upstream and...
downstream of the matched regions, and a guide tree depicting the phylogenetic relationships of these putative elements. The ends of each putative element were then determined by aligning two closely related elements with their 20 kb-flanking sequences, using NCBI-BLAST 2 SEQUENCES on the NCBI server. Usually the breakpoint of a pair-wise alignment is the boundary of a full-length element, which can be subsequently refined by identifying the terminal inverted repeats (TIRs) and TSDs around the breakpoint. Full-length elements were then classified into individual families following the guidelines proposed by Wicker et al. (2007) (Wicker et al., 2007).

Non-autonomous elements do not have coding capacity and thus, cannot be identified effectively using sequence homology-based approaches. However, virtually all plant non-autonomous cut-and-paste TEs have TIRs (10–400 bp) on both ends, are flanked by TSDs (2–10 bp), and are less than 3 kb in length. These structure features have been incorporated into an annotation pipeline MITE-Hunter (Han and Wessler, 2010), which was primarily designed to search Miniature Inverted repeat Transposable Elements (MITEs), but also can be used to search other non-autonomous cut-and-paste TEs. MITE-Hunter was employed to search the *M. guttatus* genome assembly, and the output consensus sequence for each putative nonautonomous family was used as query to retrieve all family members with 200-bp flanking sequences from the genome, using BLASTN implemented in TARGeT. Inspection of the multiple alignment profile of all family members leads to verification or refinement of the element boundary.

**Helitrons**

The program HelSearch (Yang and Bennetzen, 2009) was used to identify putative Helitron 3’ end, characterized by a CTTRR terminus that is followed by a target site T residue and is preceded 5–10 bp by a 18-bp hairpin structure. HelSearch assigns candidate 3’ ends into groups based on the hairpin stem sequences and generates multiple alignment of flanking sequences for each group. These alignments were manually inspected to define authentic 3’ end and false positives were discarded. The last 100 nucleotides of each remaining alignment of authentic 3’ end were then used as query to retrieve the 15-kb upstream sequences using TARGeT. The 5’ ends were determined by inspection of the breakpoints of pair-wise alignments of these upstream sequences using NCBI-BLAST 2 SEQUENCES on the NCBI server. A typical Helitron 5’ prime end starts with TC, following a target site A residue, and is conspicuously AT-rich in the first 18-bp region. Once the 5’ ends were identified, 70 nucleotides from both 5’ and 3’ ends for each group were joined together to form a pseudo-element, which was used as a query to retrieve all related Helitrons from the genome using TARGet, allowing the sequence between the two ends to be up to 15kb. Sequence similarity of the 70-bp region at the 5’ end was the basis for family assignment.
**LTR retrotransposons**

LTR retrotransposons were mined using LTR retrotransposons were mined using LTR_STRUC (McCarthy and McDonald, 2003), a structural data-searching program that identifies LTR retrotransposons based on the presence of the LTR pairs, the 4–6-bp TSDs flanking the LTRs, the primer binding site (PBS) and polypurine tract (PPT), as well as the canonical TG/CA dinucleotides at the 5' and 3' end of each LTR. A *copia*- and *gypsy*-specific reference sequence from the most conserved region of the reverse transcriptase (RT) domain (corresponding to Pfam family PF07727 and PF00078, respectively) was used to BLAST the full-length retrotransposon sequences found by LTR_STRUC. A phylogenetic tree was generated for all the matched *copia*-like and *gypsy*-like elements, respectively. Elements that have the RT domain were then classified into families based on the RT phylogeny. The remaining elements that do not have the RT domain (i.e. nonautonomous) were classified into families based on an all-to-all blast, following the 80-80 rule suggested by Wicker et al. (2007) (Wicker et al., 2007).

**LINEs**

The protocol of LINE identification is very similar to that of autonomous cut-and-paste TEs. There are two major clades/superfamilies of LINEs in plants, L1-like and RTE-like (Kapitonov et al., 2009). The RT amino acid sequence alignments of representative elements of these two clades were retrieved from Kapitonov et al. (2009) (Kapitonov et al., 2009) and were used as query to search all putative LINEs with their flanking sequences using TARGeT. Pair-wise alignments were then used to determine the element boundary. The 3' end is usually characterized by a poly(A) tail followed by a 7-21 bp TSD sequence. The TSD, in turn, can be used to demarcate the 5' end. The RT phylogeny was used to guide family assignments.

**SINEs**

The *M. guttatus* genome assembly was subjected to RepeatModeler open-1.0 (Smit et al., 1996), a de-novo repeat detection package that searches putative interspersed repeats and builds consensus models of identified repeat families. A TE exemplar set (see below) was built from all other elements identified by the homology-based and structure-based methods, as described above, and was used to mask the de novo repeat library output from RepeatModeler. The unmasked consensus sequences were then subjected to manual examination individually, to identify SINEs and additional TEs that were missed by structure- and homology-based methods. Sequences that are short (<500 bp) and have two conserved motifs corresponding to the Pol III internal promoter A and B boxes within the first 100-bp region are candidate SINEs (Kumar and Bennetzen, 1999; Schmidt, 1999). Each candidate
consensus sequence was used as query to retrieve all related sequences through TARGeT. A 7–21-bp TSD flanking most of these sequences would verify bono fide SINEs.

**TE examplar**

One to a few exemplar sequences were chosen to represent each family for building an exemplar library that can represent the entire TE content of the genome with a minimum number of sequences. For autonomous elements, these exemplar sequences were chosen based on two criteria: (1) sequence similarity between the exemplar and all other family members is at least 80%; (2) encoded ORFs should be the most intact. The first criterion ensures that the exemplar library can be used to mask almost all TEs in the genome prior to gene model predictions and other kinds of genome annotations. The second criterion renders convenience to use the exemplars for comparative analysis between different genomes, because elements satisfying the second criterion are often among the youngest and most abundant members of a family and the encoded protein products can be translated with minimal extent of interruption. For nonautonomous elements, only the first criterion was applied. Helitrons are much more heterogeneous in sequence between family members than other TE types, so usually many more exemplars need to be included for each family to capture the diversity. The complete *M. guttatus* TE exemplar library is available at [http://monkeyflower.uconn.edu/resources/](http://monkeyflower.uconn.edu/resources/). The TE exemplar library was used to identify TEs in the *M. guttatus* and *M. luteus* genomes using RepeatMasker ([http://www.repeatmasker.org](http://www.repeatmasker.org)).

**Accession Numbers:**

Sequence data from this article can be found in under the following accession numbers:

- *M. luteus* genome sequence, including transcript assembly, and GFF genome annotation files are available on CoGe. New version of *M. guttatus* gene annotation is available on COGE. COGE Genome IDs 22656 and 22665
- Illumina fastq reads (mate-pair and paired-end reads) used to assemble *M. luteus* genome are available on SRA (PRJNA377704)
- Gene expression data (counts and RPKM data) are available on Dryad (DOI: doi:10.5061/dryad.d4vr0)
- All called orthogroups are available Dryad (DOI: doi:10.5061/dryad.d4vr0)
- Syntenic gene sets are available Dryad (DOI: doi:10.5061/dryad.d4vr0)
- *M. guttatus* gene annotation file on Dryad (DOI: doi:10.5061/dryad.d4vr0)
- *M. luteus* gene annotation file on Dryad (DOI: doi:10.5061/dryad.d4vr0)
- *M. luteus genome* assembly file on Dryad (DOI: doi:10.5061/dryad.d4vr0)
Methylation sequencing data are available GSE95799.

Supplemental Data:
Supp. Figure 1: Chimera analysis.
Supp. Figure 2: Substitution Mean.
Supp. Figure 3: Concatenation tree.
Supp. Figure 4: Astral tree Coalescence-based phylogeny
Supp. Figure 5: Results of PUG analysis using the coalescence tree.
Supp. Figure 5: Summary of genome-wide transposon abundances
Supp. Figure 6: Relationship of TE density and gene expression for M. luteus subgenomic regions
Supp. Figure 7: Relationship of TE density and gene expression for M. guttatus subgenomic regions

ACKNOWLEDGMENTS
Funding Sources: The College of William and Mary Research Award to J.R.P., USDA-NIFA HATCH 1009804 to P.P.E., and Murdock Life Sciences Grant 2013265 to A.M.C.

AUTHOR CONTRIBUTIONS
Author contributions: Puzey and Edger designed the research; Puzey, Edger, Washburn and Pires collected samples and performed RNA-sequencing; R. Smith, G. Smith, and Puzey developed the likelihood ratio test for expression bias; Puzey, Edger, and R. Smith performed homeolog expression analyses; McKain and Edger conducted phylogenetic analyses; Puzey, Cooley, Edger, Platts, Bowman, and Childs performed genome sequencing, assembly, and annotation; Schmitz, Bewick, and Ji performed methylation analyses; Vallejo-Marin created synthetic polyploids and identified naturally occurring polyploid plants; Yuan and Puzey conducted transposon related analyses; and Puzey, Edger, and R. Smith wrote the paper.

FIGURE LEGENDS
Figure 1: Whole genome duplications in Mimulus and related species (A) Tree showing locations of whole genome duplications (stars) on Lamiales phylogeny. Coalescence-based phylogeny of 96 single copy loci estimated in ASTRAL. Node labels represent bootstrap values for 100 replicates. Nodes with bootstrap values less than 80 were collapsed. Green stars are published WGD events not identified in this study. Red stars indicate events identified in this study. Blue stars represent uncertainty in the nature of either a single event with varying support for the timing of paralog coalescence or two individual events. (B) Mimulus species used in this study. Mimulus guttatus [2x] hybridized with (C) Mimulus luteus [4x] to produce a sterile triploid (D) Mimulus robertsii [3x] which underwent a subsequent whole genome duplication giving rise to fertile natural allopolyploid (E) Mimulus peregrinus [6x]. (F) Graphic showing chromosome complement of individuals (B-E) in middle panel. The allotetraploid M. luteus has two distinct subgenomes; LA and LB represent the two
Figure 2: Expression bias of homeologs resulting from *M. luteus* specific WGD event in *M. luteus*, F1 hybrid, synthetic allopolyploid, and natural allopolyploid. Gray histograms show distribution of expression bias (ℬ) for all testable homeolog pairs. Testable homeolog pairs (N) are those which could clearly be identified as homologous and had at least 1 read in each tissue sampled. Homeolog pairs significantly biased towards the *M. guttatus*-like homeolog are crosshatched, while pairs significantly biased towards the ‘other’ homeolog are shown in solid blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the ‘other’ subgenome dominates the *M. guttatus*-like subgenome either by the number of homeologs biased towards it (NLb > NLa) or on average, |ℬlb| > |ℬla| where ℬlb and ℬla are averages over all homeolog pairs that were biased towards Lb or La, respectively.

Figure 3: Homeolog expression bias in hybrid and allopolyploids, comparing the *M. guttatus* homeolog to the weighted average expression of its pair of *M. luteus* homeologs. The average of expression of the two *M. luteus* homeologs was calculated by dividing the sum of read count of the two *M. luteus* homeologs by the sum of their individual gene lengths. Gray histograms show distribution of expression bias (ℬ) for all testable homeolog pairs. Only genes that had a clear 2 to 1 (*M. luteus* to *M. guttatus*) homology were considered. Homeolog pairs significantly biased towards the *M. guttatus* homeolog are shown in yellow, while pairs significantly biased towards the *M. luteus* homeolog are shown in blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the pair of *M. luteus* homeologs, when added together, dominates the *M. guttatus* homeolog (i.e., Nℓ > Ng and ℬℓ > ℬg, where ℬℓ and ℬg are averages over all homeolog pairs.)

Figure 4: Homeolog expression bias in hybrid and allopolyploids, comparing the *M. guttatus* homeolog to each of its *M. luteus* homeologs separately. Gray histograms show distribution of expression bias (ℬ) for all testable homeolog pairs. Homeolog pairs significantly biased towards the *M. guttatus* homeolog are shown in yellow, while pairs significantly biased towards the *M. luteus* homeolog are shown in blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the *M. luteus* homeolog dominates the *M. guttatus* homeolog (i.e., Nℓ > Ng and ℬℓ > ℬg, where ℬℓ and ℬg are averages over all homeolog pairs.)

Figure 5: Expression bias in three separate hybrid lineages. (A) Venn diagram of the number of biased homeolog pairs across hybrid lineages (1532 homeolog pairs were biased in all three lineages). (B-D) Scatter plots of expression bias (ℬ) for these 1532 homeolog pairs comparing hybrid to synthetic allopolyploid, hybrid to natural allopolyploid, and synthetic to natural allopolyploid (red line is linear regression; thin blue line is identity).

Figure 6: TE density in a window spanning 10 kb upstream to 10 kb downstream of a gene is negatively related to gene expression. The vertical axis is gene expression in RPKM. The horizontal axis is transposon density, binned into ten windows with width proportional to the number of data points it contains. TE density is negatively related to gene expression in (A) *M. guttatus* and (B) *M. luteus*. 
Figure 7: Subgenome specific methylation repatterning in hybrid and allopolyploid *Mimulus*. *M. guttatus* and *M. luteus* subgenome specific patterns of gene (top two rows) and transposon (bottom two rows) methylation. The y-axis is the weighted methylation level. The x-axis shows the gene body (TSS = transcription start site and TTS = transcription termination site) or TE body and one kilobase upstream and downstream. CG, CHG, and CHH methylation levels are shown in the first, second, and third column, respectively. Methylation levels of each individual are shown in unique colors (parents = red; F1 hybrid = light blue; synthetic allopolyploid = dark green; Natural allopolyploid = yellow).

REFERENCES


Chelaifa, H., Monnier, A., and Ainouche, M. (2010). Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species Spartina x


subgenomes in paleopolyploid cotton after 60 my of evolution. Molecular biology and
evolution 32, 1063-1071.
Renny-Byfield, S., Chester, M., Kovařík, A., Le Comber, S.C., Grandbastien, M.-A.,
sequencing reveals genome downsizing in allotetraploid Nicotiana tabacum, predominantly
through the elimination of paternally derived repetitive DNAs. Molecular biology and
evolution, msr112.
Renyi, M., Becker, C., Pélissier, T., Bogorecenik, R., Devos, J., Ikeda, Y., Weigel, D., and
Mathieu, O. (2016). Epigenome confrontation triggers immediate reprogramming of DNA
methylation and transposon silencing in Arabidopsis thaliana F1 epihybrids. Proceedings of
the National Academy of Sciences 113, E2083-E2092.
DNA. Genome research 10, 516-522.
consequences of recent hybridization and polyploidy in Spartina (Poaceae). Molecular
ecology 14, 1163-1175.
their paramutation-like interaction in tetraploid Arabidopsis thaliana. Nature genetics 34,
450-454.
Schmidt, T. (1999). LINEs, SINEs and repetitive DNA: non-LTR retrotransposons in plant
Schmitz, R.J., Schultz, M.D., Ulrich, M.A., Nery, J.R., Pelizzola, M., Libiger, O., Alix,
subgenomes by genome dominance and both ancient and ongoing gene loss. Proceedings of
the National Academy of Sciences 108, 4069-4074.
Schultz, M.D., Schmitz, R.J., and Ecker, J.R. (2012). ‘Leveling’ the playing field for
elimination and cytosine methylation are rapid and reproducible responses of the genome to
(2015). BUSCO: assessing genome assembly and annotation completeness with single-copy
orthologs. Bioinformatics, btv351.
changes in homeolog expression bias. bioRxiv, 119438.
with thousands of taxa and mixed models. Bioinformatics 22, 2688-2690.
phylogeny and the circumscription of Icacinaceae with a plastome-scale data set. American journal of botany 102, 1794-1813.


Thomas, B.C., Pedersen, B., and Freeling, M. (2006). Following tetraploidy in an Arabidopsis ancestor, genes were removed preferentially from one homeolog leaving clusters enriched in dose-sensitive genes. Genome research 16, 934-946.


Thomas, B.C., Pedersen, B., and Freeling, M. (2006). Following tetraploidy in an Arabidopsis ancestor, genes were removed preferentially from one homeolog leaving clusters enriched in dose-sensitive genes. Genome research 16, 934-946.

Thomas, B.C., Pedersen, B., and Freeling, M. (2006). Following tetraploidy in an Arabidopsis ancestor, genes were removed preferentially from one homeolog leaving clusters enriched in dose-sensitive genes. Genome research 16, 934-946.


formation has created novel avenues for evolution. Genetics 149, 1987-1996.


Figure 1: Whole genome duplications in *Mimulus* and related species (A) Tree showing locations of whole genome duplications (stars) on Lamiales phylogeny. Coalescence-based phylogeny of 96 single copy loci estimated in ASTRAL. Node labels represent bootstrap values for 100 replicates. Nodes with bootstrap values less than 80 were collapsed. Green stars are published WGD events not identified in this study. Red stars indicate events identified in this study. Blue stars represent uncertainty in the nature of either a single event with varying support for the timing of paralog coalescence or two individual events. (B) *Mimulus* species used in this study. *Mimulus guttatus* [2x] hybridized with (C) *Mimulus luteus* [4x] to produce a sterile triploid (D) *Mimulus robertsii* [3x] which underwent a subsequent whole genome duplication giving rise to fertile natural allopolyploid (E) *Mimulus peregrinus* [6x]. (F) Graphic showing chromosome complement of individuals (B-E) in middle panel. The allotetraploid *M. luteus* has two distinct subgenomes; $L_a$ and $L_b$ represent the two distinct subgenomes.
Figure 2: Expression bias of homeologs resulting from *M. luteus* specific WGD event in *M. luteus*, F1 hybrid, synthetic allopolyploid, and natural allopolyploid. Gray histograms show distribution of expression bias ($\mathcal{B}$) for all testable homeolog pairs. Testable homeolog pairs ($N$) are those which could clearly be identified as homologous and had at least 1 read in each tissue sampled. Homeolog pairs significantly biased towards the *M. guttatus*-like homeolog are crosshatched, while pairs significantly biased towards the ‘other’ homeolog are shown in solid blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the ‘other’ subgenome dominates the *M. guttatus*-like subgenome either by the number of homeologs biased towards it ($N_{L_b} > N_{L_a}$) or on average, $|\overline{\mathcal{B}}_{L_b}| > |\overline{\mathcal{B}}_{L_a}|$, where $\overline{\mathcal{B}}_{L_b}$ and $\overline{\mathcal{B}}_{L_a}$ are averages over all homeolog pairs that were biased towards $L_b$ or $L_a$, respectively.
Figure 3: Homeolog expression bias in hybrid and allopolyploids, comparing the *M. guttatus* homeolog to the weighted average expression of its pair of *M. luteus* homeologs. The average of expression of the two *M. luteus* homeologs was calculated by dividing the sum of read count of the two *M. luteus* homeologs by the sum of their individual gene lengths. Gray histograms show distribution of expression bias ($B$) for all testable homeolog pairs. Only genes that had a clear 2 to 1 (*M. luteus* to *M. guttatus*) homology were considered. Homeolog pairs significantly biased towards the *M. guttatus* homeolog are shown in yellow, while pairs significantly biased towards the *M. luteus* homeolog are shown in blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the pair of *M. luteus* homeologs, when added together, dominates the *M. guttatus* homeolog (i.e., $N_L > N_g$ and $|\bar{B}_L| > |\bar{B}_g|$, where $\bar{B}_L$ and $\bar{B}_g$ are averages over all homeolog pairs.)
Figure 4: Homeolog expression bias in hybrid and allopolyploids, comparing the *M. guttatus* homeolog to each of its *M. luteus* homeologs separately. Gray histograms show distribution of expression bias ($\mathcal{B}$) for all testable homeolog pairs. Homeolog pairs significantly biased towards the *M. guttatus* homeolog are shown in yellow, while pairs significantly biased towards the *M. luteus* homeolog are shown in blue. Across all three hybrid individuals (F1, synthetic, and natural allopolyploid) the *M. luteus* homeolog dominates the *M. guttatus* homeolog (i.e., $N_e > N_g$ and $|\mathcal{B}_e| > |\mathcal{B}_g|$), where $\mathcal{B}_e$ and $\mathcal{B}_g$ are averages over all homeolog pairs.)
**Figure 5: Expression bias in three separate hybrid lineages.** (A) Venn diagram of the number of biased homeolog pairs across hybrid lineages (1532 homeolog pairs were biased in all three lineages). (B-D) Scatter plots of expression bias ($\mathcal{B}$) for these 1532 homeolog pairs comparing hybrid to synthetic allopolyploid, hybrid to natural allopolyploid, and synthetic to natural allopolyploid (red line is linear regression; thin blue line is identity).
Figure 6: TE density in a window spanning 10 kb upstream to 10 kb downstream of a gene is negatively related to gene expression. The vertical axis is gene expression in RPKM. The horizontal axis is transposon density, binned into ten windows with width proportional to the number of data points it contains. TE density is negatively related to gene expression in (A) *M. guttatus* and (B) *M. luteus*. 
Figure 7: Subgenome specific methylation repatterning in hybrid and allopolyploid *Mimulus*. *M. guttatus* and *M. luteus* subgenome specific patterns of gene (top two rows) and transposon (bottom two rows) methylation. The y-axis is the weighted methylation level. The x-axis shows the gene body (TSS = transcription start site and TTS = transcription termination site) or TE body and one kilobase upstream and downstream. CG, CHG, and CHH methylation levels are shown in the first, second, and third column, respectively. Methylation levels of each individual are shown in unique colors (parents = red; F1 hybrid = light blue; synthetic allopolyploid = dark green; Natural allopolyploid = yellow).
Subgenome dominance in an interspecific hybrid, synthetic allopolyploid, and a 140-year-old naturally established neo-allopolyploid monkeyflower

Patrick P. Edger, Ronald D Smith, Michael R McKain, Arielle M Cooley, Mario Vallejo-Marin, Yao-Wu Yuan, Adam J Bewick, Lexiang Ji, Adrian E Platts, Megan J Bowman, Kevin Childs, Jacob D. Washburn, Robert Schmitz, Gregory D Smith, J. Chris Pires and Joshua R Puzey

Plant Cell; originally published online August 16, 2017;
DOI 10.1105/tpc.17.00010

This information is current as of March 16, 2021