THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Maternal genome dominance in early plant embryogenesis

OPEN ACCESS

Maternal genome dominance in early plant embryogenesis

Jaime Alaniz-Fabián[1#], Daoquan Xiang[2#], Gerardo Del Toro-De León[1#], Axel Orozco-Nieto[1], Peng Gao[2,3], Andrew Sharpe[3], Leon V. Kochian[3], Gopalan Selvaraj[2], Nathan Springer[4], Cei Abreu-Goodger[1], Raju Datla[2,3]*, C. Stewart Gillmor[1]*

[1]Laboratorio Nacional de Genómica para la Biodiversidad (Langebio), Unidad de Genómica Avanzada, Centro de Investigación y de Estudios Avanzados del IPN (CINVESTAV-IPN), Irapuato 36824, Guanajuato, México

[2]Aquatic and Crop Resource Development, National Research Council Canada, 110 Gymnasium Place, Saskatoon,   SK S7N 0W9, Canada

[3]Global Institute for Food Security, University of Saskatchewan, Saskatoon, SK, S7N 4J8, Canada

[4]University of Minnesota, Department of Plant and Microbial Biology, 140 Gortner Laboratory, 1479 Gortner Avenue, St. Paul, MN 55108, USA

[#]These authors contributed equally

*Corresponding authors: Raju Datla (raju.datla@gifs.ca) & C. Stewart Gillmor (stewart.gillmor@cinvestav.mx)

Keywords Arabidopsis, embryo, allele specific expression (ASE), RNA sequencing (RNAseq), zygote, zygotic genome activation, maternal-to-zygotic transition

Abstract

Previous studies have alternately supported and discounted the hypothesis that the maternal genome plays a predominant role in early embryogenesis in plants. We used 24 *embryo defective (emb)* mutants of *Arabidopsis thaliana* to test for maternal and paternal effects in early embryogenesis. 5 *emb* mutants had equal maternal and paternal effects, 5 showed maternal effects and weak paternal effects, and the remaining 14 *emb* mutants conditioned only maternal effects, demonstrating a more important role for the maternal allele for most of these *EMB* genes. To assess genome-wide maternal and paternal contributions to early embryos, we produced allele-specific transcriptomes from zygote to mature stage embryos derived from reciprocal crosses of Columbia-0 and Tsu-1, a hybrid combination we show to be a faithful proxy for isogenic Columbia-0. Parent-of-origin analysis of these transcriptomes revealed a reciprocal maternal bias in thousands of genes from the zygote to octant stage. This bias greatly diminished by the globular stage, and was absent at later stages. Comparison with egg cell transcriptomes revealed no correlation between transcript levels in the egg and maternal bias in pre-globular embryos, suggesting that the maternal bias observed in early embryos is due to preferential zygotic transcription of maternal alleles. Taken together, the functional and transcriptome data presented here support a predominant role for the maternal genome in early Arabidopsis embryogenesis.

Significance

In both animals and plants, the zygote is produced by the union of the egg and sperm cells. In animals, it is well accepted that mRNAs and proteins from the egg direct the first steps of embryogenesis. Here we present genetic and genomic experiments that support a predominant role for the maternal genome in early embryogenesis of plants, as well. In contrast to animals, our data suggest that this maternal influence is primarily derived not from inheritance of egg transcripts, but from preferential transcription of maternal alleles in the zygote and early embryo. This transient maternal zygotic bias may reflect an ancestral condition to diminish paternal influence on early embryogenesis in outcrossing plants.

Introduction

In animal embryogenesis, mRNAs and proteins inherited from the egg regulate early development until maternal products are cleared and the zygotic genome is activated, a process called the maternal to zygotic transition (Tadros & Lipshitz, 2009). In plants, most research on the maternal to zygotic transition has focused on activation of the zygotic genome. The main questions currently debated are the timing of initiation of large scale zygotic transcription, and whether this transcription initially has a maternal bias (reviewed in Del Toro-De León et al., 2016).

Initial experiments to study the onset of transcription after fertilization in wheat, maize and tobacco found novel transcripts in cDNA libraries of late zygotes compared to the egg cell (Sauter et al.,1998; Okamoto et al., 2005; Sprunck et al., 2005; Zhao et al., 2011). Recent RNA sequencing experiments comparing gametes with isogenic embryos in maize, rice and Arabidopsis have provided more evidence for transcription of a portion of the zygotic genome within hours after fertilization. In maize zygotes at 12 hours after pollination (hap) (corresponding to 4 hours after fertilization (haf) and to the G1/S phase of the cell cycle), 3605 genes were found to be upregulated compared to the egg and sperm (Chen et al., 2017). In rice, where fertilization occurs about 30 minutes after pollination, comparison of zygote and egg cell transcriptomes found 499 genes upregulated at least two-fold in zygotes at 2.5 hap (corresponding to karyogamy), 1981 genes at 5 hap (nucleolar fusion), and 2485 genes at 9 hap (G2 phase) (Anderson et al., 2017). Arabidopsis zygotes at 14 hap (approximately 6 haf) showed 2625 genes upregulated at least two-fold compared to egg cells, while at 24 hap 2951 genes were upregulated compared to egg cells (Zhao et al., 2019). In summary, these experiments show transcriptional activity for about 10% of the genome in isogenic zygotes of various plant species.

Initial experiments on maternal and paternal contributions to early isogenic embryos relied on reporter genes and functional analysis of early-acting *embryo defective (emb)* mutations. Assays of maternally and paternally-inherited reporter lines in Arabidopsis found almost 30 genes expressed primarily from the maternal allele (Vielle-Calzada et al., 2000; Autran et al., 2011; Del Toro-De León et al., 2014), as well as numerous genes with more equal maternal and paternal expression or function (Weijers et al., 2001; Lukowitz et al., 2004; Xu et al., 2005; Andreuzza et al., 2010; Aw et al., 2010; Ueda et al., 2011; Guo et al., 2016; Yang et al., 2017). In Arabidopsis, the functions of hundreds of *EMBRYO DEFECTIVE (EMB)* genes are required in early embryogenesis (Meinke, 2019). Crosses of 49 *emb* mothers to isogenic wild type plants showed that paternal alleles for 9 *EMB* genes immediately complemented lack of maternal function, while paternal alleles for 40 *EMB* genes did not fully complement until 3-5 dap (Del Toro-De León et al., 2014). Consistent with the RNA sequencing experiments mentioned above, these functional genetic experiments in isogenic embryos show that zygotic activation varies between genes, with much of the paternal genome showing less activity than the maternal genome in the first 2-3 dap.

Maternal and paternal transcripts in embryos can also be quantified using single nucleotide polymorphisms generated by crossing polymorphic ecotypes. Initial allele-specific expression (ASE) RNA sequencing (RNAseq) experiments in Arabidopsis found evidence for a transient maternal bias in early Landsberg *erecta* (L*er*) x Columbia-0 (Col) embryos (Autran et al., 2011), and for early and equal maternal and paternal contributions in Col x Cape Verde Islands (Cvi) embryos (Nodine and Bartel, 2012). Recent ASE RNAseq

3

experiments of hybrid zygotes have also included data for gametes, allowing for comparison of the egg, sperm and zygote transcriptomes. In rice, more than 97% of 14,049 genes had a strong maternal bias at both 2.5 and 9 hap, with maternal transcripts for the vast majority of genes found in both the egg and zygote (Anderson et al., 2017). In Arabidopsis, Col x L*er* hybrid zygotes had a moderate maternal bias at 14 hap (9.9% of 12,746 genes showed maternally biased transcripts), but no maternal bias by 24 hap (Zhao et al., 2019). 60% of upregulated genes at 24 hap showed biallelic expression, suggesting that maternal and paternal alleles of many genes are equally transcribed in late Col x L*er* zygotes (Zhao et al., 2019), similar to Col x Cvi embryos (Nodine and Bartel, 2012). In summary, ASE experiments in hybrid rice zygotes (Anderson et al., 2017) and Arabidopsis L*er* x Col embryos (Autran et al., 2011) argue for primarily maternal regulation of early embryogenesis, while the results of subsequent Col x L*er* (Zhao et al., 2019) and Col x Cvi (Nodine and Bartel, 2012) transcriptomes in Arabidopsis favor both maternal and paternal (i.e. zygotic) regulation of early embryogenesis. Given the differing conclusions of these studies, the extent of maternal and zygotic regulation of early embryogenesis requires further clarification.

To test whether different combinations of genetic backgrounds might explain the divergent results of hybrid transcriptomes, we previously assayed maternal and paternal effects for 11 *emb* mutants in isogenic and hybrid crosses. Maternal effects, but no paternal effects, were observed for all 11 *emb* mutants. In the embryos produced by Col *emb/+* mothers, the paternal *EMB* allele was activated significantly earlier when it was provided by L*er* or Cvi pollen compared to Wassilewskija, Tsu-1 (Tsu) or Col pollen (Del Toro-Del León et al., 2014). Here, we tested maternal and paternal effects for 24 additional *emb* mutants. These new experiments provided more evidence that maternal effects are more common than paternal effects in early embryogenesis, and that paternal allele activation is similar between the Col x Tsu hybrid and isogenic Col. We used a hand dissection protocol for isolation of zygote-1 cell to mature stage embryos from bi-directional crosses of Col x Tsu, and analyzed maternal and paternal contributions to the transcriptome. Our results demonstrate a maternal transcript bias for thousands of genes in preglobular Col x Tsu and Tsu x Col embryos, consistent with the predominant maternal effects observed in functional genetic studies with *emb* mutants. Interestingly, maternal transcript bias of genes in zygote to octant stage embryos was not correlated with the relative expression of the corresponding gene in the egg cell. Analysis of intronic reads for several hundred genes at the zygote and octant stages demonstrated that the majority of transcripts come from the maternal allele. Taken together, these results support a predominant role for the maternal genome in early embryogenesis, and suggest that this maternal dominance is due more to preferential transcription of maternal alleles in the zygote than to inheritance of maternal mRNAs from the egg cell.

Results and Discussion

Maternal effects predominate over paternal effects in early embryogenesis Maternal and paternal gametophytic effects occur in embryogenesis when the phenotype of the embryo depends on the genotype of the egg or sperm, respectively (reviewed in Armenta-Medina & Gillmor, 2019). We previously used mutations in early-acting *EMBRYO DEFECTIVE (EMB)* genes to test the activity of wt paternal alleles of 49 different *EMB* genes (Del Toro-De León et al., 2014). Because most *emb* mutants are lethal when homozygous, heterozygous (*emb/+*) plants must be used for these assays. For 40 *EMB* genes, we found that wt sperm could not complement embryos derived from mutant *emb* eggs until 3-5 dap, i.e. these 40 *emb* mutants showed a transient maternal effect. For 11 of these 40 *EMB* genes, paternal effects were tested by performing crosses using *emb/+* plants as fathers. Transient embryo phenotypes were only observed when these *emb/+* mutants were used as mothers, and not as fathers, indicating that the phenotypes were due to a maternal effect and not to haploinsufficiency (Del Toro-De León et al., 2014).

To extend this analysis, we tested 24 additional *emb* mutants for maternal and paternal effects in early embryogenesis. Phenotypes of embryos derived from hand-selfed Col *emb/+* plants, from *emb/+* mothers crossed with wt Col fathers, and from wt Col mothers crossed with *emb/+* fathers were scored at 2, 3 and 5 dap (Figure 1, Supplemental Table 1, Supplemental Data 1). All 24 *emb* mutants showed maternal effects beginning at 2 or 3 dap, which typically decreased by 5 dap (Figure 1B), and in almost all cases disappeared by 14 dap (Supplemental Table 1). The onset of paternal allele activity (i.e. a decrease in the maternal effect) can be detected as a decrease in the frequency of mutant phenotypes in embryos derived from *emb/+* x Col crosses (the father contributes two wild type alleles), compared to *emb/+* x *emb/+* crosses (the father contributes a single wild type allele). Using this criterion, paternal allele activity could first be detected at 2, 3 or 5 dap, depending on the *EMB* gene (Figure 1D & Supplemental Table 1). To determine if any of the 24 *emb* mutants also had a paternal effect, we used wt Col plants as mothers in crosses with *emb/+* fathers. Paternal effects were less common than maternal effects (compare Figures 1B&C). *nse1*, *nse3*, *miro1, iyo* and *pect1* showed transient paternal effects of the same magnitude as their transient maternal effect (Figure 1D, Supplemental Table 1). *qqt2*, *gex1*, *zyg3*, *gle1* and *fac1* showed transient paternal effects weaker than their transient maternal effects, and the remaining 14 *emb* mutants showed no paternal effect at all (Figure 1C&D & Supplemental Table 1). In summary, maternal and paternal effects were tested for 24 *emb* mutants from this study and for 11 *emb* mutants in Del Toro-De Leon et al. (2014). Maternal effects were seen for all 35 *emb* mutants, a paternal effect equal to the maternal effect was seen for 5 *emb* mutants, and a weak paternal effect was seen for an additional 5 *emb* mutants. These results show that for 30 out of 35 *EMB* genes, the maternal allele plays a more important functional role in early embryogenesis than the paternal allele.

The transient maternal and paternal effects seen for these 35 *emb* mutants could be due to a requirement for gene function in the egg, sperm, zygote, embryo, or a combination of these cell types. To try to distinguish between gametic and zygotic requirements for *EMB* function, we looked for presence of these 35 *EMB* genes in transcriptome datasets for sperm (Borges et al., 2008), eggs, zygotes and embryos (Zhao et al., 2019). 33 *EMB* genes had transcripts in zygotes and embryos, 31 of these 33 genes had egg cell transcripts, and 12 of the 33 *EMB* genes were called as present in the sperm cell transcriptome (Supplemental

Table 2). *GEX1* had a massive signal in the sperm cell, strong expression in the egg cell, and progressively decreasing transcripts in the zygote and embryo, strongly suggesting that the paternal and maternal effects observed in early *gex1/+* embryos are gametophytic (Alandete-Saez et al., 2011). *MONOPTEROS (MP)* and *WUSCHEL HOMEOBOX 2 (WOX2)*, the only genes with zygote and embryo expression but no detected egg cell transcripts, showed maternal effects but no paternal effects, suggesting that the maternal effects for these genes are zygotic maternal effects. The remaining genes had transcripts in the egg, zygote and embryo, making it impossible to distinguish between maternal gametophytic and maternal zygotic effects.

Preglobular Arabidopsis embryos show a maternal transcript bias Hybridization of polymorphic ecotypes is necessary to distinguish between RNA sequencing reads originating from the maternal and paternal genomes. However, crossing different ecotypes is known to affect parent-of-origin contributions to early embryos (Del Toro-De León et al., 2014). Notably, transient maternal effects for 11 *emb* mutants were almost identical between isogenic *emb/+* x Col and hybrid *emb/+* x Tsu crosses (Del Toro-De León et al., 2014), suggesting that parent-of-origin effects in this hybrid combination are close to the isogenic Col reference strain. To further test this hypothesis, we crossed the 24 additional *emb/+* mutants from the current study to Tsu, and compared the results with crosses to Col. For 23 of 24 *emb* mutants, transient maternal effects (i.e. *EMB* paternal allele activity) were statistically indistinguishable between *emb/+* x Col and *emb/+* x Tsu crosses (Figure 1D & Supplementary Table 1). From the combined results for the 35 *EMB* genes from this study and from Del Toro-De León et al., (2014), we conclude that the functional contributions of paternal alleles are very similar between isogenic Col and the Col x Tsu hybrid.

The similarity of paternal functional contributions between isogenic Col and Col x Tsu, and the large number of genomic DNA polymorphisms between Col and Tsu (Nordborg et al., 2005), make the Col x Tsu hybrid ideal for monitoring parent-of-origin contributions to early embryo transcriptomes. Our analysis of the Col and Tsu ecotypes identified 316,633 variants, of which 161,026 corresponded to exons in 22,741 genes (see Methods). We crossed plants of the Col and Tsu accessions in both directions (Col x Tsu and Tsu x Col), and generated transcriptomes from embryos at the zygote-1 cell stage (zyg-1cell), octant, globular, heart, torpedo, bent and mature stages. These transcriptomes exhibited several different classes of parent-of-origin bias. Genes showing reciprocal maternal bias (RMB) or reciprocal paternal bias (RPB) were biased in both directions of the cross. Genes with reciprocal Col bias (RCB) or reciprocal Tsu bias (RTB) showed higher expression of the Col or Tsu allele, respectively, regardless of the direction of the cross. No reciprocal bias (NRB) genes were those that did not show reciprocal maternal, paternal, Col or Tsu bias. General transcriptome information, complete gene expression data, and complete ASE data are found in Supplemental Data 2. ASE results for the zyg-1cell through heart stages are shown in Figure 2A, and data for all stages are presented in Figure 2B.

Thousands of genes showed reciprocal maternal bias at the zyg-1cell and octant stages (Figure 2A & B; Supplemental Data 2). In zyg-1cell embryos, 42.6% of genes showed RMB and 55.3% showed NRB (n=6,498), while octant stage embryos had 29.0% RMB genes and 69.1% NRB (n=7,510). Many RMB genes were shared between zyg-1cell and octant stage transcriptomes (Figure 2C). At the globular stage, 2.2% of genes were RMB and 95.99% NRB (n=6,723), while heart stage embryos had 0.2% RMB and 98.4% NRB genes (7,195).

6

A small percentage of genes showed paternal bias: 0.3% of genes were RPB at the zyg-1cell stage, 0.1% at the octant stage, and 0.01% at the globular stage. At the zyg-1cell stage, about 3% of genes showed reciprocal Col or Tsu bias, decreasing to less than 2% at later stages (Figure 2A; Supplemental Data 2). From heart to mature stage embryos, ASE results did not significantly vary from one stage to the next (Figure 2B; Supplemental Figure 1).

The maternal bias observed at the zyg-1cell and octant stages could be due to inheritance of transcripts from the egg, preferential transcription of maternal alleles, or both. Comparison of our data with a recent egg cell transcriptome (Zhao et al., 2019) revealed that the majority of gene transcripts in zyg-1cell and octant stage embryos are also found in egg cells: of 7,594 and 7,597 genes represented by zyg-1cell and octant embryo transcripts, 6,198 and 6,215 were present in the egg cell, respectively (Figure 2D; Supplemental Data 3). However, no correlation between the relative level of transcripts in the egg vs zyg-1cell or octant embryo, and maternal transcript bias in zyg-1cell or octant embryos was detected (Figure 2E; Supplemental Figure 2). By contrast, zyg-1cell transcripts absent from the egg (n=903) were 90.2% maternal, versus 80.8% maternal for all zyg-1cell genes; octant stage transcripts absent from the egg (n=957) were 92.0% maternal versus 78.5% for all octant genes (Figure 2F; Supplemental Data 3). The maternal bias of genes with intronic transcripts, indicative of actively transcribed genes (Gaidatzis et al., 2015), was also determined. The median maternal fraction of intronic transcripts was 97.4% in zyg-1cell (n=330 genes) and 87.7% in octant embryos (n=126 genes), while intronic reads at the globular and later stages had no maternal bias (Figure 2G; Supplemental Data 3). These results strongly suggest that the maternal transcript bias seen in zygote and octant stage embryos is at least partially due to preferential transcription of maternal alleles, and are entirely consistent with functional genetic experiments demonstrating predominantly maternal effects for *emb* mutants (Figure 1).

Embryos are surrounded by the maternally-derived seed coat, and thus contamination by maternal tissue needs to be considered when determining parent-of-origin bias in early embryo transcriptomes. An algorithm designed to identify genes enriched in embryo, endosperm and seed coat tissues detected the presence of both embryo and seed coat enriched transcripts in our zyg-1cell and octant embryo datasets (Supplemental Figure 3) (Schon & Nodine, 2017). To test if the maternal bias we observed in our zyg-1cell and octant transcriptomes was influenced by maternal sporophytic transcripts, we used ovule transcriptomes to calculate zyg-1cell/ovule and octant/ovule expression ratios on a gene-by-gene basis. Transcripts for genes enriched ten times in zyg-1cell embryos compared to the ovule (n=129) were 81.3% maternal, compared to a median maternal ratio of 80.8% for all zygotic genes (n=8673). Transcripts for genes enriched ten times in octant embryos compared to the ovule (n=138) were 85.2% maternal, compared to a median 78.5% maternal bias for all octant embryo transcripts (n=8093) (Figure 2F & Supplemental Data 3). We also compared the maternal bias for all genes at the zyg-1cell and octant stages with their embryo/ovule expression ratio. No correlation was found between maternal bias and zyg-1cell/ovule or octant/ovule expression ratio (Supplemental Figure 4 & Supplemental Data 3). These results demonstrate that genes with higher expression in the ovule than the embryo show no more maternal bias than genes which are higher in the embryo than the ovule. We conclude that contamination by seed coat transcripts does not have a significant effect on the overall maternal transcript bias observed in zyg-1cell and octant embryos.

7

To further validate the transcriptome results above, we checked for correlations between transcriptome data and functional or reporter gene data. *emb* mutants with both maternal and paternal effects are likely to be haploinsuficient in early embryogenesis, and would be expected to have similar levels of maternal and paternal transcripts, while *emb* mutants with only maternal effects must have maternal expression and may or may not have similar levels of paternal expression. Of the 35 *EMB* genes with functional data from this study or Del Toro-De León et al. (2014), 19 are represented by parent-of-origin data in the Col x Tsu transcriptome (Supplemental Table 2). Of these 19 *EMB* genes, the five *emb* mutants with both maternal and paternal effects (*iyo*, *nse3*, *qqt2*, *fac1* and *gle1*) have similar amounts of maternal and paternal reads, while the 14 *emb* mutants with only maternal effects have maternal reads with varying ratios of paternal reads (Supplemental Table 2). Parent-of-origin expression for three reciprocally maternally biased genes is also consistent with reporter gene data. *NUCLEAR FACTOR Y SUBUNIT B2 (NF-YB2)* showed almost exclusively maternal transcripts at the zyg-1cell and octant stages, and strong maternal transcript bias at the globular stage, in agreement with strictly maternal expression of an *pNF-YB2::GUS* marker (Siefers et al., 2009) at 2 and 3 dap (Figure 3 & Supplemental Table 3). *GRAND CENTRAL* (*GCT*) and *CENTER CITY (CCT)* also showed reciprocal maternal transcript bias at the zyg-1cell and octant stages, in agreement with *gGCT-GUS* and *pCCT::GUS* reporters which showed maternally biased GUS marker expression at 2 and 3 dap (Del Toro-De León et al., 2014; Supplemental Table 3). The correlation between transcriptome, functional and marker data is consistent with faithful representation of parent-of-origin biases in the transcriptome reported here.

In contrast to the maternal transcript bias in Col x Tsu embryos at the zyg-1cell and octant stage, equal maternal and paternal transcripts were found in 1-2 cell and octant Col x Cvi embryos (Nodine & Bartel, 2012), and Col x L*er* zygotes (Zhao et al., 2019). The Col x Tsu, Col x Cvi, and Col x L*er* hybrid combinations were previously found to be divergent for paternal allele activation (Del Toro-De León et al., 2014), suggesting that the different parent-of-origin transcriptome contributions found in these three hybrids reflect biological differences between the ecotypes used. For example, the equal maternal and paternal transcripts in Col x Cvi may be due to the unique chromatin status of the Cvi ecotype, which has genome-wide hypomethylation at CG sites (Schmitz et al., 2013; Pignatta et al., 2014), and more open chromatin (Tessadori et al., 2009), leading to an increase in transcription in Col x Cvi hybrids (Baroux et al., 2013). The predominance of maternal effects observed in analysis of *emb* mutants in Col and Col x Tsu embryos, and the maternal transcript bias observed in Col x Tsu embryos, may also have an epigenetic basis. The Arabidopsis egg cell lacks expression of the CG maintenance methyltransferase *MET1* (Jullien et al., 2012), and has low levels of CG methylation, likely due to hemi-methylation of CG sites (Ingouff et al., 2017). By contrast, the Arabidopsis sperm cell has steady levels of CG methylation (Ingouff et al., 2017). When these parental genomes are united in the zygote, the resulting asymmetry in CG methylation could result in greater transcriptional silencing of the paternal genome than the maternal genome, due to silencing proteins like SU(VAR)3-9 HOMOLOG2 (SUVH2) and METHYL BINDING DOMAIN 5 (MBD5), MBD6 & MBD7 that specifically bind to symmetrically methylated CpG sites to (Johnson et al., 2008; Zemach & Grafi, 2003). The variation in CG methylation that exists throughout the genome could explain why parent-of-origin bias varies between genes, and might underlie some of the differences observed between ecotypes, given the variation in DNA methylation that exists between Cvi and other

accessions (Schmitz et al., 2013; Pignatta et al., 2014). A recent study in rice found large differences in siRNA populations of eggs and sperm (Li et al., 2020), which could influence transcription of maternal and paternal genomes in rice zygotes (Anderson et al., 2017).

Changes in mRNA metabolism between ecotypes or hybrids could also explain differences in transcript populations in early embryos. A transcriptome of L*er* x Col embryos that used random primers to generate libraries for sequencing found a maternal bias in early embryos (Autran et al., 2011). Significant differences between total mRNA and polyA mRNA populations are known in animal zygotes (Aanes et al., 2011), have previously been found in maize (Grimanelli et al., 2005), and are very likely to exist in Arabidopsis as well. For example, mRNAs for *WOX2*, one of the most important transcription factors in early embryogenesis in Arabidopsis, have been detected in egg cells by *in situ* hybridization (Haecker et al., 2004), but are absent from an egg cell transcriptome produced using oligodT primers (Supplemental Table 2; Zhao et al., 2019), suggesting that the increase in *WOX2* transcripts detected by RNAseq after fertilization is at least partly due to polyadenylation of existing *WOX2* mRNAs.

Conclusion

The genetic and transcriptome data reported here show a predominant role for the maternal genome during early Arabidopsis embryogenesis, and point to Col x Tsu as an opportune hybrid for studies of parent-of-origin gene regulation in Arabidopsis. Why might parent-of-origin transcript ratios vary between different hybrids? The parental conflict theory would predict maternal silencing of paternal alleles to be adaptive (Baroux & Grossniklaus, 2015). However, in an inbred species like *Arabidopsis thaliana*, relative changes in early maternal and paternal transcription may have less functional relevance than they would in an outcrossing species, allowing for rapid evolution in parent-of-origin gene expression in seeds of different ecotypes (Geist et al., 2019). For example, the RNA-dependent DNA methylation pathway, as well as histone methylation and histone replacement, have been demonstrated to regulate expression of paternal alleles in early Arabidopsis embryos (Autran et al., 2011). Yet loss of function phenotypes of these pathways have only subtle phenotypes during embryogenesis (Pillot et al., 2010; Autran et al., 2011). By contrast, loss of the maternal RdDM pathway in the outcrossing species *Brassica rapa* causes severe phenotypes during reproduction (Grover et al., 2018), demonstrating the importance of maternal RdDM regulation of seed development in a close relative of Arabidopsis. A better understanding of the mechanisms that regulate maternal and paternal transcript ratios in the zygote and early embryo will reveal more about the biological role that maternal dominance plays in early embryogenesis in *Arabidopsis thaliana*, and will facilitate comparative studies of maternal and paternal regulation of embryogenesis in species of agronomic importance like rice and maize.

Methods

In notation of crosses, the mother is always listed first. *emb/+* crosses and reporter gene studies (NF-YB2 reporter ABRC stock CS67020) were conducted as in Del Toro-De León et al. (2014). To produce hybrid embryos for ASE analysis, the inflorescence of the female parent was pruned to leave only the two oldest flower buds, which were pollinated 4 hours after emasculation. Embryos from reciprocal crosses of Col-0 (ABRC stock CS60000) and Tsu-1 (ABRC stock CS6926) were collected and processed as in Xiang et al. (2011). Zygote and octant stage embryos were washed in the dissection solution by moving away from ovule and endosperm tissue and then pipetting into eppendorf tubes on dry ice. Globular and later stage embryos were washed using several changes of dissection solution. ~300 zygote and 1-cell stage embryos (referred to as zyg-1cell stage) were collected at 32 hours after pollination (32 hap), ~200 octant embryos at 60 hap, ~100 globular embryos at 72 hap, and ~100 heart, ~60 torpedo, ~30 bent and ~30 mature embryos, based on morphological stage, as well as ~10 unfertilized Col ovules. Two biological replicates were collected for all stages in both directions, except for Col x Tsu zyg-1cell stage (one biological replicate). Raw data from Tsu-1 genome re-sequencing (Ossowski et al., 2008) was used to call variants regarding Col-0 (TAIR 10). Reads were trimmed from both ends to have a mean phred quality of at least 30 in 4bp windows using fastp V 0.19.6 (Chen et al., 2018); they were then aligned against Col-0 genome (TAIR 10) using the Burrows-Wheeler aligner V 0.7.17-r1188 (Li & Durbin, 2009) with the aln algorithm, allowing up to 10% of read length as edit distance (-n 0.1); after sorting and marking duplicates with sambamba V 0.6.8 (Tarasov et al., 2015), potential variants were detected using freebayes V 1.0.0 (Garrison and Marth, 2012). Variants were called if they had a minimum phred quality of 20 and a maximum of 0.05 normalized likelihood of being heterozygous. Diploid genome and annotation for the hybrid were generated by substituting these variants into the TAIR10 genome assembly and Araport 11 annotation (Cheng et al., 2017).

For quantification of transcript abundance, reads were aligned against the diploid genome using STAR V 2.7.0d (Dobin et al., 2013), with the following flags: --alignIntronMax 900 --alignMatesGapMax 900. Reads aligned to genes were then counted using featureCounts V 1.6.0 (Liao et al., 2014) with the following flags: -M -p -O –fraction. Counts for both alleles were summed for each gene, and converted to reads per million (RPM). Abundance ratios between samples were calculated only for genes with at least 20 RPM in at least one of the samples and after adding 5 RPM to the abundance in each tissue, to avoid infinite ratios, and to stabilize the ratios in low-abundance transcripts. To analyze the overlap of present transcripts between stages, genes with more than 10 RPM were considered present and genes with less than 1 RPM were considered absent; genes with transcript levels between 1 and 10 RPM at any stage were ignored, to minimize false call rate. For all comparisons between Col x Tsu embryos and the isogenic Col-0 egg or ovule, only ASE data for the Col x Tsu cross was used, to avoid confounding effects from Tsu maternal transcripts of dissimilar abundance between ecotypes.

For quantification of allele-specific transcript abundance, STAR was run again as previously described, plus the following flags: --outFilterMultimapNmax 1 --outFilterMultimapScoreRange 0. Then, only reads aligned to called variants in genes were counted; a reduced annotation which included only the variable regions of each gene was used, and featureCounts was run as above but without the -M flag; this produced a table of counts for each allele for each gene. For the statistical analysis of allele-specific differential

abundance we used the edgeR package (Robinson et al., 2010). Only genes with at least 3 RPM aligned to variants in at least 2 samples were considered for the differential expression analysis. The sum of counts for both alleles was used to calculate library sizes and normalization factors. Tagwise dispersion and the likelihood ratio test were used to obtain FDR-adjusted P values and fold change estimates, which were converted to allelic ratio estimates. For all representations of allelic ratios, all genes with at least 20 reads aligned to variants, considering the sum across replicates, were used. A cutoff of FDR<= 0.05 was used to call significant allelic biases. For analysis of intronic reads, reads were counted only if they aligned to a variant in an intron that did not overlap with any exon of any isoform in the Araport 11 annotation. To diminish the impact of unknown isoforms from the Tsu accession, only reads for the Col x Tsu cross were used. Technical replicates were summed at the count level and treated as a single sample. Raw sequence data are archived in the GEO database (GSE132449).

Acknowledgements

References

Aanes H, et al. (2011) Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. *Genome Res* 21(8):1328–1338.

Alandete-Saez M, Ron M, Leiboff S, McCormick S (2011) Arabidopsis thaliana *GEX1* has dual functions in gametophyte development and early embryogenesis. *Plant J* 68(4):620–632.

Anderson SN, et al. (2017) The Zygotic Transition Is Initiated in Unicellular Plant Zygotes with Asymmetric Activation of Parental Genomes. *Dev Cell* 43(3):349–358.e4.

Andreuzza S, et al. (2010) DNA LIGASE I exerts a maternal effect on seed development in Arabidopsis thaliana. *Development* 137(1):73–81.

Armenta-Medina A, Gillmor CS (2019) Genetic, molecular and parent-of-origin regulation of early embryogenesis in flowering plants. *Curr Top Dev Biol* 131:497–543.

Autran D, et al. (2011) Maternal epigenetic pathways control parental contributions to Arabidopsis early embryogenesis. *Cell* 145(5):707–719.

Aw SJ, Hamamura Y, Chen Z, Schnittger A, Berger F (2010) Sperm entry is sufficient to trigger division of the central cell but the paternal genome is required for endosperm development in Arabidopsis. *Development* 137(16):2683–2690.

Borges F, et al. (2008) Comparative transcriptomics of Arabidopsis sperm cells. *Plant Physiol* 148(2):1168–1181.

Baroux C, Autran D, Raissig MT, Grimanelli D, Grossniklaus U (2013) Parental contributions to the transcriptome of early plant embryos. *Curr Opin Genet Dev* 23(1):72–74.

Baroux C, Grossniklaus U (2015) The Maternal-to-Zygotic Transition in Flowering Plants: Evidence, Mechanisms, and Plasticity. *Curr Top Dev Biol* 113:351–371.

Chen J, et al. (2017) Zygotic genome activation occurs shortly after fertilization in maize. *Plant Cell* 29(9):2106–2125.

Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34(17):i884–i890.

Cheng C-Y, et al. (2017) Araport11: a complete reannotation of the Arabidopsis thaliana reference genome. *Plant J* 89(4):789–804.

Del Toro-De León G, Garcia-Aguilar M, Gillmor CS (2014) Non-equivalent contributions of maternal and paternal genomes to early plant embryogenesis. *Nature* 514(7524):624–627.

Del Toro-De León G, Lepe-Soltero D, Gillmor CS (2016) Zygotic genome activation in isogenic and hybrid plant embryos. *Curr Opin Plant Biol* 29:148–153.

Dobin A, et al. (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1):15–21.

Gaidatzis D, Burger L, Florescu M, Stadler MB (2015) Analysis of intronic and exonic reads in RNA-seq data characterizes transcriptional and post-transcriptional regulation. *Nat Biotechnol* 33(7):722–729.

Garrison, E, Marth, G (2012) Haplotype-based variant detection from short-read sequencing. https://arxiv.org/abs/1207.3907v2

Geist KS, Strassmann JE, Queller DC (2019) Family quarrels in seeds and rapid adaptive evolution in Arabidopsis. *Proceedings of the National Academy of Sciences* 116(19):9463–9468.

Grimanelli D, Perotti E, Ramirez J, Leblanc O (2005) Timing of the maternal-to-zygotic transition during early seed development in maize. *Plant Cell* 17(4):1061–1072.

Grover JW, et al. (2018) Maternal components of RNA-directed DNA methylation are required for seed development in Brassica rapa. *Plant J* 94(4):575–582.

Guo L, et al. (2016) The anaphase-promoting complex initiates zygote division in Arabidopsis through degradation of cyclin B1. *Plant J* 86(2):161–174.

Haecker A, et al. (2004) Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. *Development* 131(3):657–668.

Ingouff M, et al. (2017) Live-cell analysis of DNA methylation during sexual reproduction in Arabidopsis reveals context and sex-specific dynamics controlled by noncanonical RdDM. *Genes Dev* 31(1):72–83.

Johnson LM, Law JA, Khattar A, Henderson IR, Jacobsen SE (2008) SRA-domain proteins required for DRM2-mediated de novo DNA methylation. *PLoS Genet* 4(11):e1000280.

Jullien PE, Susaki D, Yelagandula R, Higashiyama T, Berger F (2012) DNA Methylation Dynamics during Sexual Reproduction in *Arabidopsis thaliana*. *Curr Biol* 22(19):1825–1830.

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754–1760.

Li C, et al. (2020) Genome-wide redistribution of 24-nt siRNAs in rice gametes. *Genome Res*:gr.253674.119.

Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30(7):923–930.

Lukowitz W, Roeder A, Parmenter D, Somerville C (2004) A MAPKK kinase gene regulates extra-embryonic cell fate in Arabidopsis. *Cell* 116(1):109–119.

Meinke DW (2019) Genome-wide identification of *EMBRYO-DEFECTIVE (EMB)* genes required for growth and development in Arabidopsis. *New Phytol*. doi 10.1111/nph.16071

Nodine MD, Bartel DP (2012) Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. *Nature* 482(7383):94–97.

Nordborg M, et al. (2005) The pattern of polymorphism in *Arabidopsis thaliana*. *PLoS Biol* 3(7):e196.

Okamoto T, Scholten S, Lörz H, Kranz E (2005) Identification of genes that are up- or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygote development by a cell manipulation- and PCR- based approach. *Plant Cell Physiol* 46:332-338.

Ossowski S, et al. (2008) Sequencing of natural strains of Arabidopsis thaliana with short reads. *Genome Res* 18(12):2024–2033.

Pignatta D, et al. (2014) Natural epigenetic polymorphisms lead to intraspecific variation in Arabidopsis gene imprinting. *Elife* 3:e03198.

Pillot M, et al. (2010) Embryo and endosperm inherit distinct chromatin and transcriptional states from the female gametes in Arabidopsis. *Plant Cell* 22(2):307–320.

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1):139–140.

Sauter M, von Wiegen P, Lörz H, Kranz E (1998) Cell cycle regulatory genes from maize are differentially controlled during fertilization and first embryonic cell division. *Sex Plant Reprod* 11:41-48.

Schmitz RJ, et al. (2013) Patterns of population epigenomic diversity. *Nature* 495(7440):193–198.

Schon MA, Nodine MD (2017) Widespread Contamination of Arabidopsis Embryo and Endosperm Transcriptome Data Sets. *Plant Cell* 29(4):608–617.

Siefers N, et al. (2009) Tissue-Specific Expression Patterns of Arabidopsis NF-Y Transcription Factors Suggest Potential for Extensive Combinatorial Complexity. *Plant Physiol* 149(2):625–641.

Sprunck S, Baumann U, Edwards K, Langridge P, Dresselhaus T (2005) The transcript composition of egg cells changes significantly following fertilization in wheat (*Triticum aestivum L.*). *Plant J* 41:660-672.

Tadros W, Lipshitz HD (2009) The maternal-to-zygotic transition: a play in two acts. *Development* 136(18):3033–3042.

Tarasov A, Vilella AJ, Cuppen E, Nijman IJ, Prins P (2015) Sambamba: fast processing of NGS alignment formats. *Bioinformatics* 31(12):2032–2034.

Tessadori F, et al. (2009) *PHYTOCHROME B* and *HISTONE DEACETYLASE 6* Control Light-Induced Chromatin Compaction in *Arabidopsis thaliana*. *PLoS Genet* 5(9):e1000638.

Ueda M, Zhang Z, Laux T (2011) Transcriptional activation of Arabidopsis axis patterning genes WOX8/9 links zygote polarity to embryo development. *Dev Cell* 20(2):264–270.

Vielle-Calzada JP, Baskar R, Grossniklaus U (2000) Delayed activation of the paternal genome during seed development. *Nature* 404(6773):91–94.

Weijers D, Geldner N, Offringa R, Jürgens G (2001) Seed development: Early paternal gene activity in Arabidopsis. *Nature* 414(6865):709–710.

Xiang D, et al. (2011) Genome-wide analysis reveals gene expression and metabolic network dynamics during embryo development in Arabidopsis. *Plant Physiol* 156(1):346–356.

Xu J, et al. (2005) EMBRYONIC FACTOR 1 encodes an AMP deaminase and is essential for the zygote to embryo transition in Arabidopsis. *Plant J* 42(5):743–756.

Yang K-J, Guo L, Hou X-L, Gong H-Q, Liu C-M (2017) ZYGOTE-ARREST 3 that encodes the tRNA ligase is essential for zygote division in Arabidopsis. *J Integr Plant Biol* 59(9):680–692.

Zemach A, Grafi G (2003) Characterization of *Arabidopsis thaliana* methyl- CpG- binding domain (MBD) proteins. *Plant J* 34(5):565–572.

Zhao J, Xin H, Qu L, Ning J, Peng X, Yan T, Ma L, Li S, Sun M-X (2011) Dynamic changes of transcript profiles after fertilization are associated with de novo transcription and maternal elimination in tobacco zygote, and mark the onset of the maternal-to-zygotic transition. *Plant J* 65:131-145.

Zhao P, et al. (2019) Two-Step Maternal-to-Zygotic Transition with Two-Phase Parental Genome Contributions. *Dev Cell* 49(6):882–893.e5.
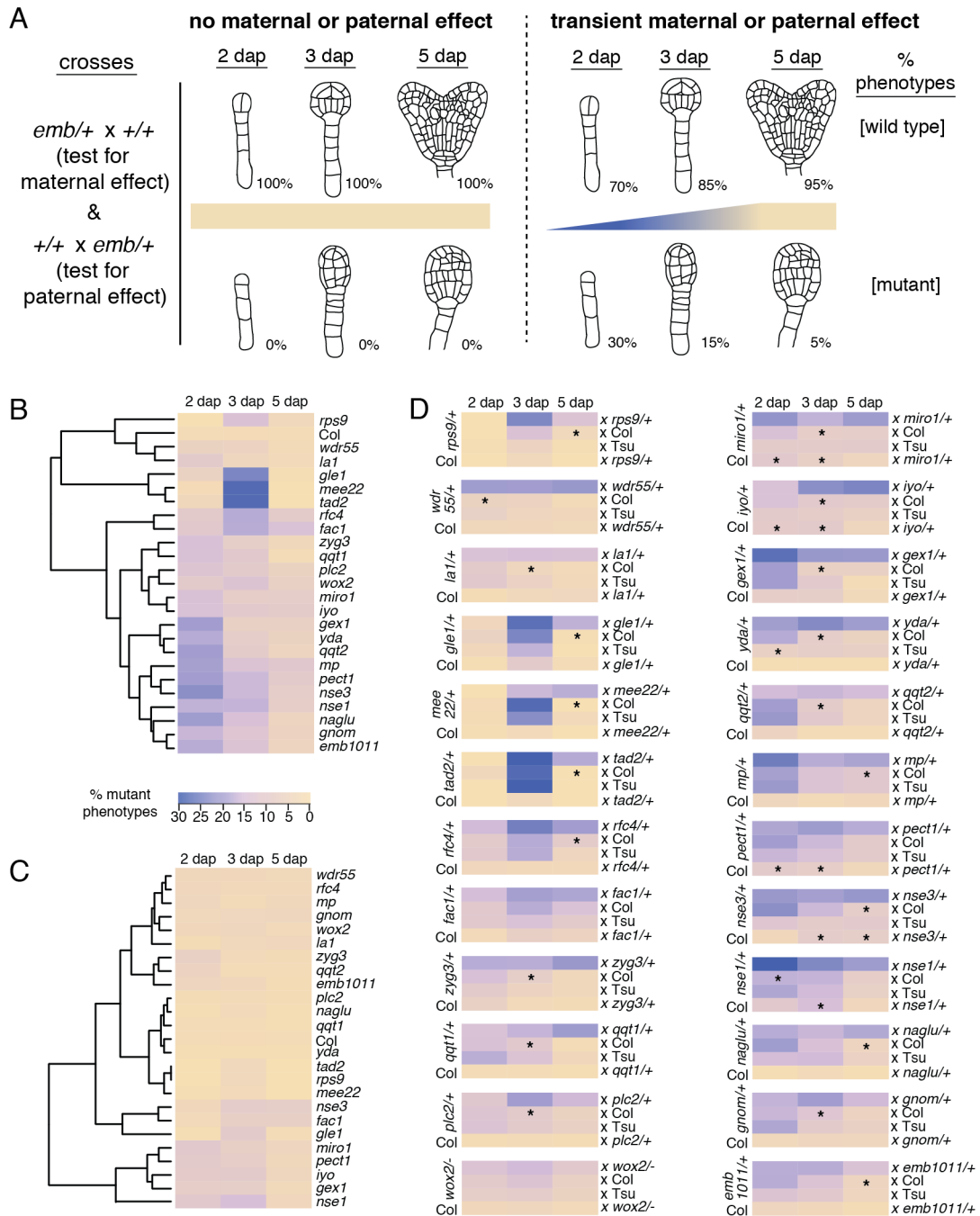
Figure 1. Maternal effects of *emb* mutants are more common than paternal effects, and are similar between isogenic Col and Col x Tsu hybrid embryos (A) Schematic representations of results for crosses to test maternal and paternal effects of *emb* mutants. Left, percentage of wild type and mutant phenotypes in the absence of maternal and paternal effects. Right, hypothetical example of penetrance of mutant phenotypes when maternal or paternal effects are seen. (B) Maternal effects observed in embryos from *emb/+* x Col crosses at 2, 3, and 5 dap. (C) Paternal effects observed in embryos from Col x *emb/+* crosses at 2, 3, and 5 dap. (D) Mutant phenotypes observed in embryos of *emb/+* x *emb/+*; *emb/+* x Col; *emb/+* x Tsu; and Col x *emb/+* plants at 2, 3, and 5 dap. For *emb/+* x Col crosses, the asterisk denotes the first stage where a significant decrease in mutant phenotypes could be observed compared with *emb/+* x *emb/+* crosses. For *emb/+* x Tsu crosses, the asterisk marks a significant difference from *emb/+* x Col. For Col x *emb/+* crosses, the asterisk denotes stages with both a significant difference with Col x Col control crosses, and no significant difference with *emb/+* x Col crosses (i.e. equal maternal and paternal effects). Statistical significance $p<0.05$, determined by Fisher's two-tailed test. The intensity of purple denotes the % of mutant phenotypes observed in embryos resulting from the indicated crosses. See Supplemental Table 1 and Supplemental Data 1 for full information.

16
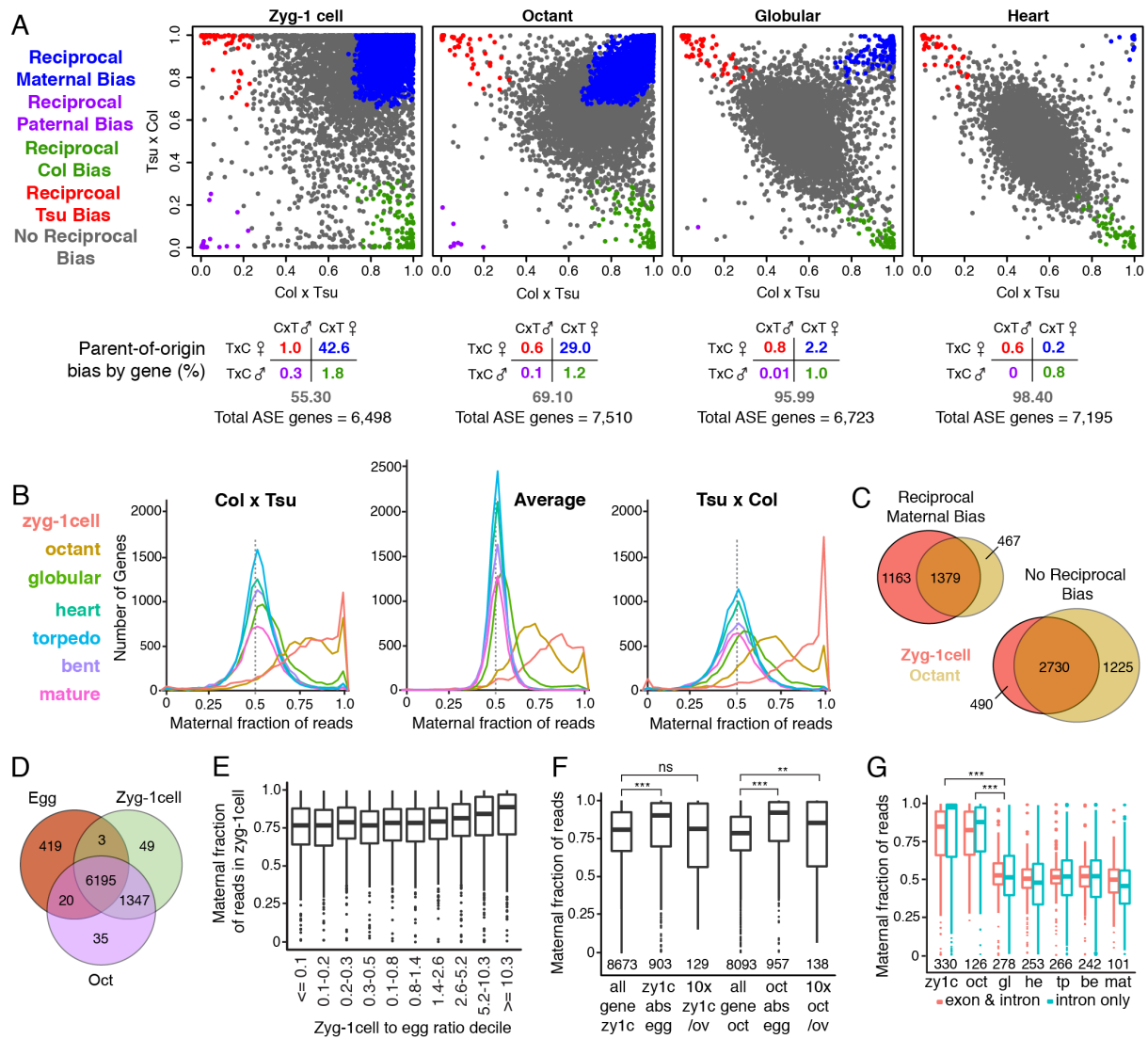
Figure 2. Maternal transcripts predominate in preglobular embryos (A) Top, maternal fractions of reads for genes in embryos from Col x Tsu and Tsu x Col crosses, at the zyg-1cell, octant, globular and heart stages, where 1.0 is exclusively maternal and 0.0 is exclusively paternal. RMB, reciprocal maternal bias; RPB, reciprocal paternal bias; RCB, reciprocal Col bias; RTB, reciprocal Tsu bias; NRB, no reciprocal bias. Bottom, percentage of genes in each reciprocal bias category, or with no reciprocal bias (grey). (B) Number of genes corresponding to maternal fraction of reads at the zyg-1cell, octant, globular, heart, torpedo, bent and mature stages, in Col x Tsu embryos, Tsu x Col embryos, and the average of the reads for each gene between the two crosses. (C) Venn diagram for the overlap of reciprocal maternal bias and no reciprocal bias genes at the zyg-1cell (orange) and octant (tan) stages. (D) Venn diagram for genes with transcripts present in the egg, zyg-1cell and octant embryo. (E) Maternal fraction of reads for genes at the zyg-1cell stage, based on their zyg-1cell to egg expression ratio, separated into deciles. The Pearson's value for the correlation of maternal fraction with zyg-1cell to egg ratio is 0.07 ($p=2.7e^{-7}$) (F) Maternal fraction of reads for all genes expressed in zyg-1cell embryos; genes expressed at the zyg-1cell and absent from egg ($p=3.03e^{-15}$); genes expressed ten times higher at the zyg-1cell embryo than in the ovule ($p=9.31e^{-3}$); all genes expressed in the octant embryo; genes expressed in the octant embryo and absent from the egg ($p=2.19e^{-22}$); and genes expressed ten times higher in the octant embryo compared to the ovule ($p=1.03e^{-02}$). (G) Maternal fraction of reads for genes with reads mapping to introns, and reads mapping to exons and introns, at the zyg-1cell through mature stages. Zyg-1cell exon & intron reads vs globular exon & intron reads ($p=4.9e^{-49}$). Zyg-1cell intron reads vs globular intron reads ($p=4.06e^{-36}$). Octant exon & intron reads vs globular exon & intron reads ($p=5.63e^{-32}$). Octant intron reads vs globular intron reads ($p=1.84e^{-24}$). p values for results of a Wilcox test comparing the maternal fraction of reads between samples are listed in parentheses. ***, p < 0.001; ** p<0.01

17
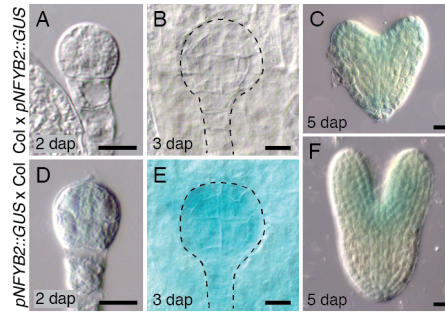
Figure 3. Maternally biased expression of *pNF-YB2::GUS* marker Expression of *pNF-YB2::GUS* from paternal (A-C) and maternal (D-F) alleles is shown at 2, 3 and 5 dap. See Supplemental Table 3 for quantification of GUS expression. Scale bar = 10µm.
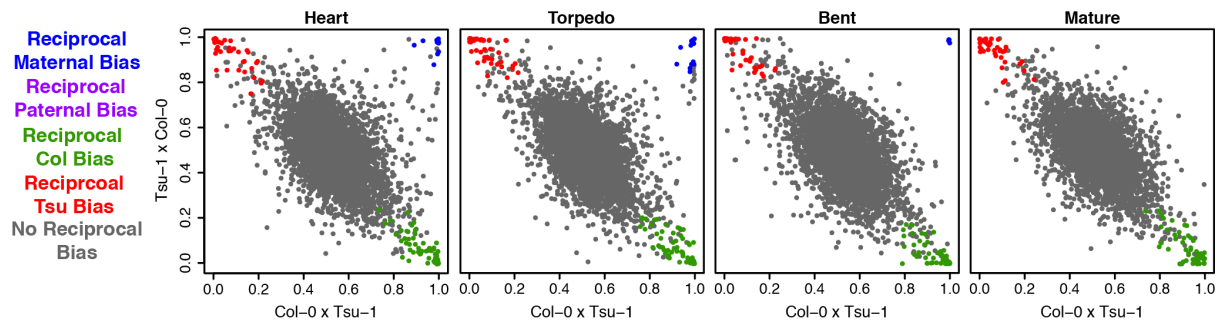
Supplemental Figure 1. Maternal fraction of reads for genes at the heart, torpedo, bent and mature stages Maternal fraction of reads for genes in embryos from Col x Tsu and Tsu x Col crosses, where 1.0 is exclusively maternal and 0.0 is exclusively paternal. RMB, reciprocal maternal bias; RPB, reciprocal paternal bias; RCB, reciprocal Col bias; RTB, reciprocal Tsu bias; NRB, no reciprocal bias.
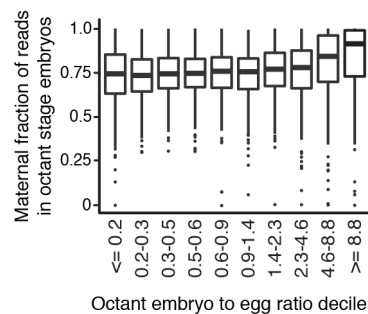
Supplemental Figure 2. Maternal fraction of reads for genes compared to their octant embryo to egg expression ratios Maternal fraction of reads in the octant embryo for genes based on their octant embryo to egg expression ratio, separated into deciles. The Pearson's value for the correlation of maternal fraction with zygote to egg ratio is 0.08 (p=6.7e^{-10}).

18
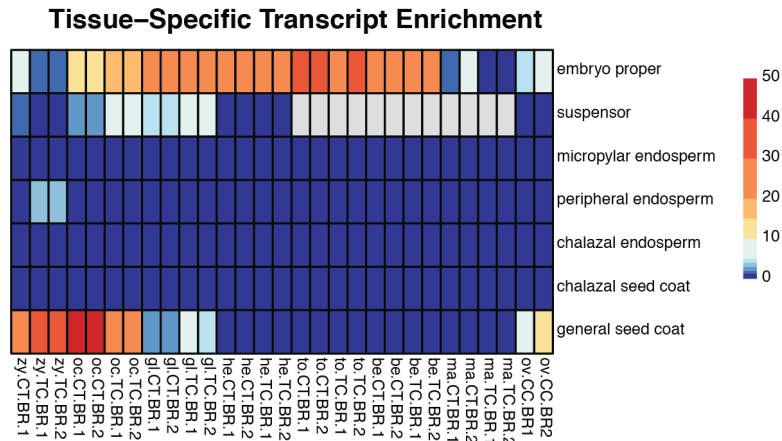
Supplemental Figure 3. Results of Nodine and Schon tissue enrichment test Total mapped reads (without regard to parent-or-origin) were run on https://github.com/Gregor-Mendel-Institute/ tissue-enrichment-test.
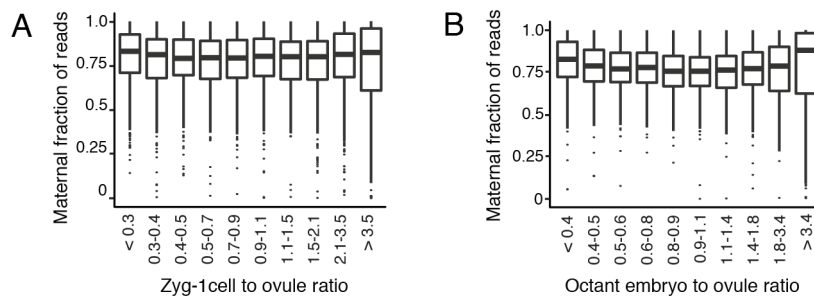
Supplemental Figure 4. Maternal fraction of reads for genes based on their zyg-1cell and octant stage embryo to ovule expression ratio (A) Maternal fraction of reads for genes classified into deciles by the ratio of their zyg-1cell to ovule expression (Pearson's correlation = 0.01, *p*=0.48) (B) Maternal fraction of reads for genes classified into deciles by the ratio of their octant stage embryo to ovule expression (Pearson's correlation = 0.03, *p*=0.02).

Supplemental Table 1. Functional genetic analysis of transient maternal and paternal effects in early Arabidopsis embryogenesis. For each *embryo defective (emb)* mutant, the results of hand self pollinations, crosses to wild type Columbia (Col-0) (CS1092) and Tsu (Tsu-1) (CS28782), are shown. Maternal genotype in crosses is listed first. *emb/+* (heterozygous) plants were used in all crosses, except for *wox2*, which is homozygous viable and was used as a homozygote. Results are the sum of at least three different siliques from each of two plants. Statistically significant p values for Fisher's Two-Tailed Exact Test are shaded in grey. ***p<0.001; ** p<0.01; * p<0.05.

AtG Identifier, Gene, Allele and ABRC stock number	Cross	2 dap % mut	mut	norm	p vs x Col	p vs x Tsu	p vs Col x Col	3 dap % mut	mut	norm	p vs x Col	p vs x Tsu	p vs Col x Col	5 dap % mut	mut	norm	p vs x Col	p vs x Tsu	p vs Col x Col	14 dap % mut	mut	norm
At1g21690	rfc4/+ x rfc4/+	16.08%	50	261	0.0578	0.241	2.57e-09***	25.37%	85	250	0.0529	0.0981	2.15e-18***	20.78%	69	263	0.000154***	1.48e-06***	5.02E-14	19.85%	26	105
REPLICATION FACTOR C4 (RFC4) / EMB1968	rfc4/+ x Col	10.21%	24	211		0.729	0.00015***	18.38%	43	191		1	5.88e-11***	9.76%	28	259		0.0648	0.000053	1.79%	2	110
rfc4-1 / emb1968	rfc4/+ x Tsu	11.28%	15	118	0.729			18.18%	26	117	1			4.61%	7	145	0.0648					
CS16161	Col x rfc4/+	3.97%	10	242	0.00751**	0.0083**	0.29	2.96%	9	295	1.59e-09***	1.05e-07***	0.4	2.99%	9	292	0.000992**	0.423	0.428	1.80%	2	109
Unknown	emb1011/+ x emb1011/+	18.15%	55	248	1	0.292	8.19e-11***	18.21%	57	256	0.106	0.0799	1.16e-11***	14.07%	37	226	0.0003***	0.00542**	6.72E-08	12.40%	15	106
EMB1011	emb1011/+ x Col	18.18%	62	279		0.3	3.89e-11***	13.51%	45	288		0.631	3.76e-08***	5.30%	17	304		1	0.03	1.63%	2	121
emb1011-1	emb1011/+ x Tsu	13.21%	14	92	0.3			11.11%	13	104	0.631			4.76%	6	120	1			0.69%	1	143
CS16007	Col x emb1011/+	4.60%	15	311	2.24e-08***	0.00582**	0.109	3.62%	13	346	3.28e-06***	0.004**	0.21	1.62%	6	365	0.00952**	0.0842	1	0.69%	1	143
At5g22370	qqt1/+ x qqt1/+	14.13%	51	310	1	0.316	4.39e-08***	17.01%	58	283	0.0315*	0.405	5.19e-11***	21.19%	75	279	4.48e-17***	1.29e-06***	7.84E-15	26.62%	41	113
QUATRE-QUART 1 / EMB1705	qqt1/+ x Col	14.32%	56	335		0.323	2.34e-08***	11.32%	43	337		0.535	0.00000139*	1.98%	7	347		0.477	1	0.58%	1	170
emb1705-2	qqt1/+ x Tsu	17.97%	23	105	0.323			13.43%	18	116	0.535			3.45%	4	112	0.477			1.18%	2	167
SALK_059852	Col x qqt1/+	1.96%	7	350	2e-10***	3.7e-09***	1	1.97%	7	348	2.21e-07***	2.34e-06***	1	0.83%	3	360	0.218	0.0622	0.295	1.18%	2	167
At4g21800	qqt2/+ x qqt2/+	15.94%	51	269	0.136	0.211	3.13e-09***	16.73%	43	214	0.0157*	0.248	6.24e-10***	15.83%	41	218	5.38e-05***	0.00233**	4.05E-09	12.82%	20	136
QUATRE-QUART 2	qqt2/+ x Col	20.51%	73	283		0.775	2.14e-13***	9.57%	29	274		0.699	0.0000433**	5.17%	14	257		1	0.059	0.99%	1	100
qqt2	qqt2/+ x Tsu	21.74%	20	72	0.775			11.11%	11	88	0.699			4.96%	6	115	1					
SALK_026078	Col x qqt2/+	5.57%	16	271	1.92e-08***	2.1e-05***	0.042*	1.79%	5	275	5.53e-05***	0.000318***	1	1.87%	5	262	0.0591	0.105	1	0.00%	0	154
At2g38280	fac1/+ x fac1/+	15.73%	45	241	0.174	0.285	8.22e-09***	19.84%	49	198	0.716	0.34	2.88e-12***	19.14%	40	169	0.225	0.135	9.06E-11	16.84%	16	79
ADENOSINE 5'-MONOPHOSPHATE DEAMINASE / EMBRYONIC FACTOR 1 (FAC1)	fac1/+ x Col	11.48%	31	239		1	0.000016**	18.18%	36	162		0.501	3.16e-10***	14.36%	26	155		0.531	3.73E-07	1.11%	1	89
fac1-2	fac1/+ x Tsu	11.38%	14	109	1			14.44%	13	77	0.501			10.77%	7	58	0.531					
SALK_055006C	Col x fac1/+	2.82%	8	276	8.45e-05***	0.00117**	0.585	7.66%	21	253	0.000863**	0.0621	0.000898**	7.65%	14	169	0.0451*	0.443	0.0035	0.96%	1	103
At5g21140	nse1/+ x nse1/+	30.57%	107	243	6.12e-05***	0.0116*	1.30e-22***	23.96%	81	257	0.036*	0.0654	4.09e-17***	21.20%	74	275	5.53e-06***	0.000161***	1.29E-14	23.70%	41	132
NON-SMC ELEMENT 1 (NSE1) / EMB 1379	nse1/+ x Col	17.49%	60	283		0.691	1.43e-10***	17.16%	58	280		0.892	4.74e-11***	8.36%	25	274		0.85	0.00052	4.83%	7	138
nse1-1 / emb1379	nse1/+ x Tsu	18.94%	25	107	0.691			16.06%	22	115	0.892			7.30%	10	127	0.85			2.33%	3	126
CS16151	Col x nse1/+	11.31%	38	298	0.0286*	0.0352*	0.0000079*	15.20%	52	290	0.532	0.889	2.34e-09***	3.94%	14	341	0.0202*	0.159	0.161	2.33%	3	126
At1g13980	gnom/+ x gnom/+	17.36%	50	238	0.912	0.774	3.90e-10***	20.38%	54	211	0.0162*	0.016*	8.17e-13***	16.18%	44	228	3.4e-05***	0.021*	1.71E-09	16.94%	21	103
GNOM / EMB30	gnom/+ x Col	16.67%	48	240		0.565	1.37e-09***	12.62%	38	263		0.631	4.02e-07***	5.45%	17	295		0.491	0.028	1.42%	2	139
emb30-1	gnom/+ x Tsu	18.97%	22	94	0.565			10.53%	14	119	0.631			7.14%	8	104	0.491			0.68%	1	146
CS6320	Col x gnom/+	3.32%	11	320	1.2e-08***	3.6e-07***	0.444	4.21%	12	273	0.000307***	0.0167*	0.124	4.38%	14	306	0.328	0.316	0.102	0.68%	1	146
At5g27540	miro1/+ x miro1/+	22.33%	23	80	0.161	0.0133*	1.66e-09***	17.39%	20	95	0.0356*	0.0896	8.12e-08***	21.30%	23	85	0.00849**	0.0183*	1.36E-09	20.97%	26	98
MIRO RELATED GTPASE 1 (MIRO1) / EMB2473	miro1/+ x Col	14.29%	17	102		0.308	0.0000094*	8.33%	11	121		0.828	0.00355**	8.59%	11	117		0.831	0.00418	1.02%	1	97
miro1-1 / emb2473	miro1/+ x Tsu	9.43%	10	96	0.308			9.68%	12	112	0.828			10.00%	13	117	0.831			0.69%	1	143
CS16182	Col x miro1/+	11.29%	14	110	0.565	0.672	0.000234**	7.44%	9	112	0.82	0.65	0.0064**	4.80%	6	119	0.316	0.153	0.11	0.69%	1	143
At5g13690	naglu/+ x naglu/+	18.32%	24	107	0.64	0.618	3.17e-08***	16.78%	24	119	0.74	1	3.35e-08***	18.70%	23	100	0.000779***	0.00587**	1.23E-08	18.58%	21	92
N-ACETYLGLUCOSIMINIDASE (NAGLU) / CYCLOPS 1 (CYL1)	naglu/+ x Col	21.09%	27	101		0.328	9.28e-10***	14.73%	19	110		0.856	0.00000107**	5.15%	7	129		0.784	0.115	0.77%	1	129
naglu	naglu/+ x Tsu	15.70%	19	102	0.328			16.04%	17	89	0.856			6.42%	7	102	0.784			2.22%	2	88
SALK_065889C	Col x naglu/+	1.47%	2	134	1.25e-07***	3.63e-05***	1	2.67%	4	146	0.000301***	0.000273***	0.479	1.22%	2	162	0.0841	0.0322*	0.7144723	2.22%	2	88
At1g63700	yoda/+ x yoda/+	21.07%	55	206	0.509	0.00153**	1.04e-12***	23.77%	63	202	4.76e-06***	0.000711***	1.06e-15***	21.12%	53	198	5.11e-07***	0.00185**	2.67E-13	20.90%	28	106
YODA (YDA) / EMB71	yoda/+ x Col	18.39%	48	213		0.0081**	1.34e-10***	8.76%	22	229		1	0.000384**	6.08%	16	247		0.643	0.0139	6.02%	8	125
emb71	yoda/+ x Tsu	7.14%	7	91	0.0081**			8.57%	9	96	1			7.55%	8	98	0.643					
CS84618	Col x yoda/+	1.26%	3	235	1.18e-11***	0.00805**	0.725	1.23%	3	240	0.000114***	0.00147**	1	1.28%	3	232	0.00479**	0.00481**	0.729	2.40%	3	122
At1g19850	mp-12/+ x mp-12/+	25.66%	88	255	0.0662	0.435	9.78e-18***	18.22%	49	220	0.197	0.415	3.23e-11***	21.91%	71	253	0.000495***	0.0272*	4.38E-15	19.85%	26	105
AUXIN RESPONSE FACTOR 5 (ARF5) / MONOPTEROS (MP)	mp-12/+ x Col	19.64%	65	266		0.673	2.27e-12***	13.30%	25	163		1	8.68e-07***	11.55%	38	291		1	0.0000018	2.27%	3	129
mp-12	mp-12/+ x Tsu	21.57%	22	80	0.673			13.64%	12	76	1			11.58%	11	84	1					
SALK_149553	Col x mp-12/+	4.28%	16	358	9.32e-11***	3.03e-07***	0.173	2.46%	6	238	1.71e-05***	0.000281***	0.539	3.83%	13	326	0.000209***	0.00848**	0.227	2.19%	3	134
AT5G59340	wox2-1/- x wox2-1/-	13.50%	37	237	0.383	0.142	4.65e-07***	16.07%	36	188	0.359	0.117	5.23e-09***	11.50%	26	200	0.198	0.0963	0.0000085	10.77%	7	58
WUSCHEL RELATED HOMEOBOX 2 (WOX2)	wox2-1/- x Col	10.91%	37	302		0.499	0.0000142*	13.03%	31	207		0.359	5.52e-07***	6.98%	9	120		0.806	0.0166	1.03%	1	96
wox2-1	wox2-1/- x Tsu	8.27%	11	122	0.499			9.09%	9	90	0.359			6.06%	8	124	0.806					
Obtained from Thomas Laux	Col x wox2-1/-	3.54%	9	245	0.000938***	0.0547	0.417	4.44%	10	215	0.00159**	0.124	0.1005	2.74%	6	213	0.0975	0.16	0.552	0.00%	0	114
At5g55490	gex1/+ x gex1/+	27.36%	29	77	0.301	0.262	1.67e-12***	21.88%	28	100	0.00106**	0.0138*	7.12e-11***	21.55%	25	91	0.00154**	1.65e-06***	4.51E-10	21.93%	25	89
GAMETE EXPRESSED PROTEIN 1 (GEX1)	gex1/+ x Col	20.25%	16	63		1	2.24e-07***	6.96%	8	107		0.48	0.0115*	7.20%	9	116		0.0624	0.0152	5.83%	6	97
gex1-1	gex1/+ x Tsu	20.18%	22	87	1			9.82%	11	101	0.48			1.75%	2	112	0.0624					

Locus / Line	Cross	%	n	N	p	p	p	%	n	N	p	p	p	%	n	N	p	p	p	%	n	N
CS817261	Col x gex1/+	8.33%	11	121	0.0181*	0.00868**	0.0054**	7.94%	10	116	0.811	0.652	0.0035**	3.36%	4	115	0.256	0.684	0.465	3.25%	4	119
AT4G38440	iyo/+ x iyo/+	14.63%	18	105	1	0.503	.0000051	23.15%	25	83	0.00999**	0.00508**	8.30e-11***	24.30%	26	81	0.0037**	0.000371***	2.71E-11	23.85%	26	83
MINIYO (IYO)	iyo/+ x Col	14.56%	15	88		0.488	.000015**	9.80%	10	92		1	0.00095***	9.48%	11	105		0.633	0.00129	5.21%	5	91
iyo-2	iyo/+ x Tsu	10.14%	7	62	0.488			8.82%	9	93	1			7.02%	8	106	0.633					
SALK_099872	Col x iyo/+	9.30%	12	117	0.224	1	0.0024**	10.17%	12	106	1	0.82	0.00038***	4.46%	5	107	0.195	0.57	0.166	1.71%	2	115
At4g32720	atla1/+ x atla1/+	14.16%	16	97	0.425	0.686	.0000145	15.38%	18	99	0.00739**	0.0346*	8.86e-07***	14.29%	18	108	0.00217**	0.0046**	0.0000033	15.97%	19	100
AtLA1	atla1/+ x Col	10.43%	12	103		0.831	0.00077***	4.42%	5	108		0.566	0.143	2.68%	3	109		1	0.697	1.92%	2	102
atla1-1	atla1/+ x Tsu	11.54%	12	92	0.831			6.36%	7	103	0.566			2.97%	3	98	1					
CS823259	Col x atla1/+	1.67%	2	118	0.00504**	0.0039**	1	3.28%	4	118	0.741	0.358	0.446	4.03%	5	119	0.725	0.733	0.298	0.76%	1	130
At2g34260	wdr55/+ x wdr55/+	21.10%	23	86	0.00136**	0.00593**	4.42e-09***	20.37%	22	86	0.00192**	0.000401***	3.14e-09***	21.85%	26	93	7.02e-07***	2.06e-05***	2.26E-10	21.05%	28	105
WD REPEAT DOMAIN 55 (WDR55)	wdr55/+ x Col	6.03%	7	109		0.792	0.0569	5.56%	6	102		0.768	0.072	2.29%	3	128		0.709	0.72	0.87%	1	114
wdr55-2	wdr55/+ x Tsu	7.34%	8	101	0.792			4.59%	5	104	0.768			3.48%	4	111	0.709					
CS855491	Col x wdr55/+	3.79%	5	127	0.556	0.261	0.321	3.57%	3	81	0.734	1	0.373	3.42%	5	141	0.726	1	0.331	0.00%	0	143
AT1G07910	zyg3/+ x zyg3/+	18.35%	20	89	0.59	0.2	1.33e-07***	18.10%	19	86	0.0417*	0.00527**	7.04e-08***	21.82%	24	86	7.32e-05***	6.81e-05***	5.61e-10	20.72%	23	88
ARABIDOPSIS THALIANA RNA LIGASE (ATRNL) / ZYGOTE ARREST 3 (ZYG3)	zyg3/+ x Col	15.04%	17	96		0.498	.0000052**	8.18%	9	101		0.594	0.00388**	3.81%	4	101		1	0.274	2.86%	3	102
zyg3-2	zyg3/+ x Tsu	10.61%	7	59	0.498			5.56%	6	102	0.594			3.74%	4	103	1					
CS746016	Col x zyg3/+	6.94%	10	134	0.0414*	0.417	0.0247	2.90%	4	134	0.0853	0.342	0.461	2.03%	3	145	0.454	0.458	1	1.61%	2	122
AT1G34770	nse3/+ x nse3/+	21.74%	20	72	0.868	0.0908	1.06e-08***	19.82%	22	89	0.605	0.0372*	4.91e-09***	22.00%	22	78	0.00747**	0.0301*	1.25E-09	20.79%	21	80
NON-SMC ELEMENT 3 (NSE3)	nse3/+ x Col	23.01%	26	87		0.0543	2.57e-10***	16.52%	19	96		0.12	2.43e-07***	8.77%	10	104		0.812	0.0028	3.45%	4	112
nse3-2	nse3/+ x Tsu	12.50%	14	98	0.0543			9.40%	11	106	0.12			9.89%	9	82	0.812					
CS334183	Col x nse3/+	3.64%	4	106	1.87e-05***	0.0246*	0.463	9.26%	10	98	0.116	1	0.00138**	7.96%	9	104	1	0.63	0.00652	0.83%	1	119
AT2G38670	pect1/+ x pect1/+	18.92%	21	90	0.867	0.719	5.74e-08***	21.28%	20	74	0.378	0.259	4.02e-09***	18.28%	17	76	0.146	0.149	2.32E-07	14.85%	15	86
PHOSPHORYLETHANOLAMINE CYTIDYLYLTRANSFERASE 1 (PECT1)	pect1/+ x Col	20.18%	23	91		0.594	9.40e-09***	16.38%	19	97			2.72e-07***	10.20%	10	88			0.00109	5.56%	5	85
pect1	pect1/+ x Tsu	16.67%	16	80	0.594			14.58%	14	82	0.849			10.53%	10	85	1					
SALK_085102	Col x pect1/+	10.10%	10	89	0.0569	0.209	0.00175**	8.33%	8	88	0.0986	0.257	0.00479**	3.85%	4	100	0.0977	0.0946	0.272	0.85%	1	117
At1g48175	emb2191/+ x emb2191/+	0.00%	0	107	0.06	1	0.327	36.00%	56	96	0.09	0.319	1.25e-22***	17.50%	24	106	3.4e-10***	0.000000247***	0.0000000104***	nd		
TRNA ADENOSINE DEAMINASE 2/ EMB2191	emb2191/+ x Col	3.60%	5	130		0.06	0.327	27.30%	49	125		0.097	5.90e-17***	0.00%	0	175		1	0.161	nd		
emb2191-1	emb2191/+ x Tsu	0.00%	0	122	0.06		0.177	30.40%	42	94	0.61		3.30e-17***	0.00%	0	106	1		0.327	nd		
CS16170	Col x emb2191/+	0.00%	0	111	0.06	0.06	0.328	3.10%	4	122	3.1e-9***	3.15e-09***	0.449	0.00%	0	109	1	1	0.327	nd		
At1g13120	emb1745/+ x emb1745/+	4.10%	6	140	0.58	1	0.221	27.40%	45	119	0.61	0.043*	5.01e-16***	16.40%	23	108	1.5e-7***	8.27e-08***	3.39e-08***	nd		
GLE1/EMB1745	emb1745/+ x Col	6.10%	8	120		0.394	0.038*	24.20%	39	121		0.165	1.14e-13***	1.10%	2	170		0.50753	0.7102427	nd		
emb1745	emb1745/+ x Tsu	3.30%	5	139	0.39		0.506	16.80%	27	128	0.16		7.22e-09***	0.00%	0	131	0.5		0.1773198	nd		
CS16088	Col x emb1745/+	0.68%	1	141	0.01**	0.213	0.425	9.50%	12	106	0.002**	0.115	0.00038***	1.60%	2	113	1	0.217521	0.6717021	nd		
At1g74970	twn3/+ x twn3/+	0.00%	0	135	1	0.48	0.167	24.10%	30	94	0.07	0.000299	3.71e-12***	15.50%	15	97	0.006**	0.0000278***	0.0000194**	nd		
RIBOSOMAL PROTEIN S 9 (RPS9)/TWIN3 (TWN3)	twn3/+ x Col	0.00%	0	152		0.451	0.161	13.00%	11	69		0.113	0.00005347***	3.00%	4	121		0.19	0.473	nd		
twn3	twn3/+ x Tsu	0.80%	1	124	0.45		0.668	5.60%	5	82	0.11		0.0524	0.70%	1	140	0.19		0.668	nd		
CS24062	Col x twn3/+	0.00%	0	159	1	0.44	0.161	3.00%	5	153	0.004**	0.332	0.316	0.00%	0	109	0.12	1	0.327	nd		
At2g34780	emb1611/+ x emb1611/+	0.00%	0	108	0.5	0.498	0.327	16.50%	20	101	0.02*	0.253	1.65e-07***	17.90%	23	105	6.4e-7***	0.00011***	2.34e-08***	nd		
MATERNAL EFFECT EMBRYO ARREST 22 (MEE22)/EMB1611	emb1611/+ x Col	1.50%	2	124		1	1	28.10%	43	110		0.402	4.65e-16***	0.00%	0	101		0.248	0.328799	nd		
emb1611	emb1611/+ x Tsu	1.60%	2	116	1		1	23.20%	27	89	0.4		3.04e-11***	2.70%	3	108	0.2		0.696	nd		
CS16110	Col x emb1611/+	0.80%	1	123	1	0.614	0.668	2.50%	3	113	5.3e-9***	0.00000212***	0.683	0.00%	0	85	1	0.259	0.342	nd		
AT3G08510	plc2/+ x plc2/+	12.00%	15	110	1	0.714	0.000102***	20.77%	27	103	0.0246*	0.0383*	2.90e-10***	16.22%	18	93	0.0289*	0.0367*	6.97e-07***	17.39%	20	95
PHOSPHOLIPASE C2 (PLC2)	plc2/+ x Col	12.77%	18	123		0.86	0.000024***	10.08%	13	116		1	0.00031***	6.12%	6	92			0.075	0.83%	1	119
plc2-2	plc2/+ x Tsu	13.64%	18	114	0.86		0.0000116***	10.83%	13	107	1		0.000173***	6.96%	8	107	1		0.0253*			
SALK_152284	Col x plc2/+	1.65%	2	119	0.000687**	0.000306**	1	2.46%	3	119	0.0181*	0.00961**	0.687	1.68%	2	117	0.145	0.0563	1	1.72%	2	114
	Col x Col	2%	5	247				1.60%	4	247				1.90%	5	264				1.70%	5	282
	Col x Tsu	2.80%	4	137				2.70%	4	144				0.80%	1	131				nd		

Statistically significant p values for Fisher's Two-Tailed Exact Test are shaded in grey. ***p<0.001; ** p<0.01; * p<0.05.

Supplemental Table 2. Comparison of transient maternal and paternal effects with egg, zygote and embryo transcriptome data. Maternal and paternal effect data are from this study (1) and from Del Toro-De León et al., 2014 (2). Egg, zygote and embryo expression data (FPKM) from isogenic Col are from Zhao et al., 2019. Sperm cell expression data (ATH1 microarray) and presence (P) absence (A) calls from isogenic Col are from Borges et al., 2008. Col x Tsu hybrid transcriptome data are from this study, and represent data from the Col x Tsu cross only, to make the data most directly comparable with egg and zygote transcriptome data in the Col ecotype, and functional analysis in the Col ecotype. NMB = no maternal bias (maternal fraction adjusted p value > 0.05); Mat = maternal bias (maternal fraction adjusted p value < 0.05).

AtG designation	Gene name	Maternal effect	Paternal effect	Egg cell	Zygote 14h	Zygote 24 h	1 cell embryo	32 cell embryo	Sperm cell signal	Sperm cell call	Col x Tsu Zyg-1cell	Col x Tsu Octant	Col x Tsu Globular
AT2G34260	*WDR55* (1)	Weak	No	156	165	161	189	135	19	A	NMB	NMB	NMB
AT5G21140	*NSE1 / EMB1379* (1)	Yes	Yes	139	81	14	39	27	-	-	-	-	-
AT5G27540	*MIRO1 / EMB2473* (1)	Yes	Yes	37	65	56	74	59	69	A	-	-	-
AT4G38440	*IYO* (1)	Yes	Yes	11	14	12	15	5	53	A	NMB	NMB	NMB
AT1G34770	*NSE3* (1)	Yes	Yes	23	34	47	68	59	254	P	NMB	NMB	NMB
AT2G38670	*PECT1* (1)	Yes	Yes	92	115	133	114	132	24	A	-	-	-
AT4G21800	*QQT2* (1)	Yes	Weak	38	59	42	57	56	38	A	NMB	NMB	NMB
AT2G38280	*FAC1* (1)	Yes	Weak	67	52	54	67	43	33	A	NMB	NMB	NMB
AT5G55490	*GEX1* (1)	Yes	Weak	163	113	6	1	0	10753	P	-	-	-
AT1G07910	*ZYG3* (1)	Yes	Weak	8	11	5	7	6	1147	P	-	-	-
AT1G13120	*GLE1 / EMB1745* (2)	Yes	Weak	27	47	42	30	48	630	P	NMB	NMB	NMB
AT1G48175	*EMB2191* (1)	Yes	No	36	30	43	36	19	-	-	Mat	Mat	NMB
AT1G74970	*RPS9 / TWN3* (1)	Yes	No	25	14	8	41	53	16	A	Mat	Mat	NMB
AT2G34780	*MEE22 / EMB1611* (1)	Yes	No	16	17	14	17	11	27	A	NMB	NMB	NMB
AT1G21690	*RFC4 / EMB1968* (1)	Yes	No	40	48	115	92	139	492	P	-	-	-
AT5G22370	*QQT1 / EMB1705* (1)	Yes	No	49	45	53	96	62	15	A	-	-	-
AT1G13980	*GNOM / EMB30* (1)	Yes	No	7	7	7	10	8	2847	P	Mat	Mat	NMB
AT5G13690	*NAGLU* (1)	Yes	No	12	11	13	9	15	87	A	-	-	-
AT1G63700	*YDA* (1)	Yes	No	34	63	48	35	19	36	A	Mat	NMB	NMB
AT1G19850	*MP* (1)	Yes	No	0	1	0	21	130	11	A	-	-	-
AT5G59340	*WOX2* (1)	Yes	No	0	4	229	104	87	69	A	NMB	NMB	NMB
AT4G32720	*AtLA1* (1)	Yes	No	115	237	149	242	214	350	P	-	-	-
AT3G08510	*PLC2* (1)	Yes	No	9	24	35	14	28	844	P	Mat	Mat	NMB
unknown	*EMB1011* (1)	Yes	No	-	-	-	-	-	-	-	-	-	-
AT2G38020	*VCL1* (2)	Yes	No	41	31	16	12	12	18	A	-	-	-
AT5G15920	*SMC5 / EMB2782* (2)	Yes	No	25	40	25	30	16	76	P	NMB	NMB	NMB
AT3G10220	*TFC B / EMB2804* (2)	Yes	No	33	37	50	52	78	-	-	-	-	-
AT1G55900	*TIM50 / EMB1860* (2)	Yes	No	97	99	52	53	64	28	A	NMB	NMB	NMB
AT1G67320	*EMB2813* (2)	Yes	No	8	33	31	38	57	1310	P	NMB	NMB	NMB
AT1G08840	*EMB2411* (2)	Yes	No	11	13	13	8	8	45	A	-	-	-
AT5G27740	*RFC3 / EMB2775* (2)	Yes	No	62	80	70	92	85	91	P	NMB	Mat	NMB
AT1G10270	*GRP23* (2)	Yes	No	35	30	13	38	16	40	A	Mat	Mat	NMB
AT1G49880	*EMB3106* (2)	Yes	No	9	9	26	10	9	-	-	-	-	-
AT2G32590	*EMB2795* (2)	Yes	No	2	4	8	33	12	111	P	Mat	NMB	NMB
unknown	*EMB2421* (2)	Yes	No	-	-	-	-	-	-	-	-	-	-

Supplemental Table 3. Comparison of maternal transcript ratios and reporter gene expression for the reciprocal maternal bias genes *NUCLEAR FACTOR Y SUBUNIT B2 (NF-YB2)*, *GRAND CENTRAL (GCT)*, and *CENTER CITY (CCT)*. Data for Col x Tsu (CxT) and Tsu x Col (TxC) transcriptomes at the zyg-1cell, octant globular and heart stages are from Supplemental Dataset 1; p value indicates probability of maternal bias. GUS reporter data shown as fraction of embryos with GUS expression for pNF-YB2::eGFP/GUS line (ABRC germplasm CS67020) (this study), and pCCT::GUS and gGCT-GUS (Del Toro-De León et al., 2014) at 1, 2, 3, and 5 dap; p value for Fisher's Two Tailed Test indicates whether maternal and paternal GUS expression ratios are different from each other. Statistically significant maternal ratios are highlighted in grey.

Gene ID	Gene name	Zyg-1cell				Octant				Globular				Heart				2 dap					3 dap					5 dap				
		CxT	p	TxC	p	CxT	p	TxC	p	CxT	p	TxC	p	CxT	p	TxC	p	GUS x Col	n	Col x GUS	n	p	GUS x Col	n	Col x GUS	n	p	GUS x Col	n	Col x GUS	n	p
At5g47640	*NF-YB2*	0.974	1E-09	0.991	3E-24	0.971	5E-16	0.92	2E-09	0.782	0.004	0.828	0.004	0.672	0.467	0.425	0.964	0.77	53	0	129	2E-30	0.89	91	0	137	4E-51	0.76	140	0.71	137	0.41
At1g55325	*GCT*	0.897	6E-05	0.904	2E-08	0.905	4E-07	0.826	6E-04	0.577	0.769	0.712	0.322	0.543	1	0.522	1	0.84	237	0	118	6E-61	0.77	121	0.61	271	0.002	1	99	1	72	1
At4g00450	*CCT*	0.778	0.017	0.716	0.013	0.832	9E-05	0.713	0.036	0.622	0.511	0.451	0.948	0.51	1	0.581	0.93	0.92	166	0	133	1E-70	0.82	147	0.56	293	2E-08	0.99	106	1	99	0.38