Supporting Information for Florez-Rueda *et al.*

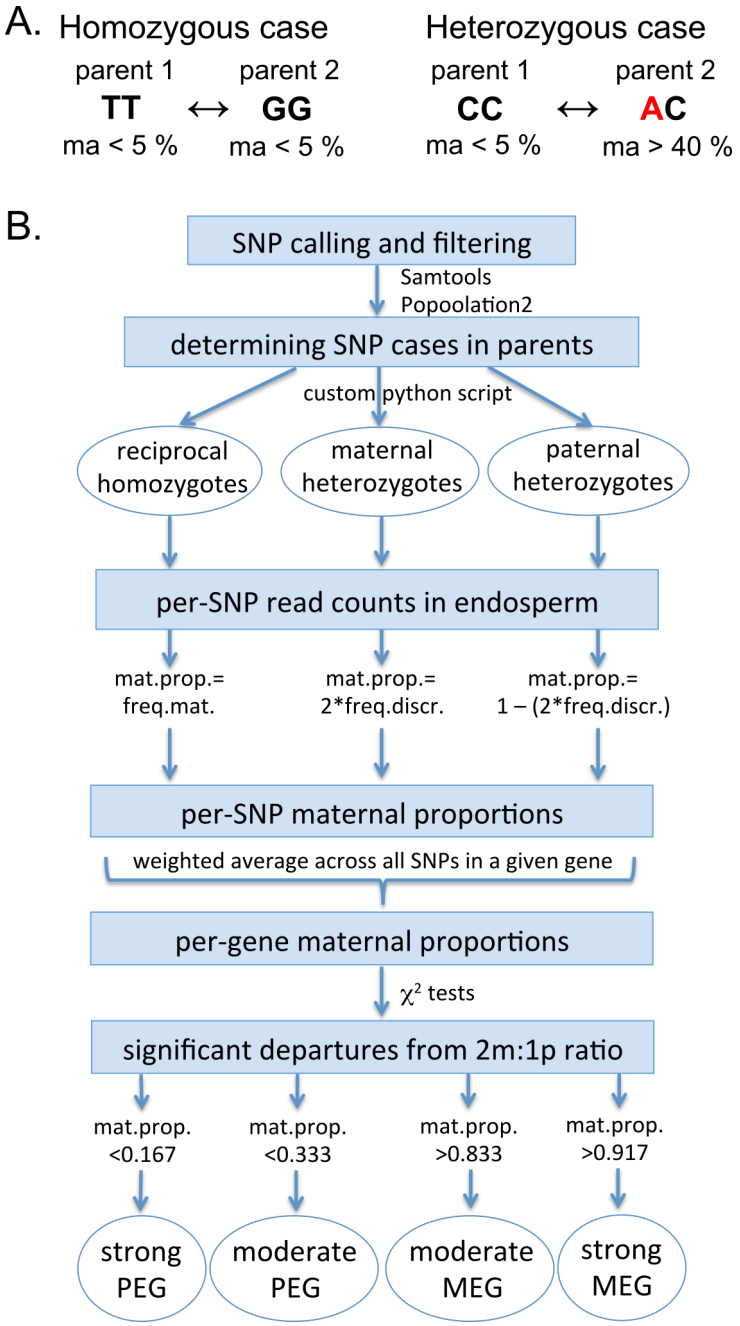

FIG. S1. Diagram showing the pipeline implemented in our custom Python program. (*A*) Identification of homozygous and heterozygous differences (SNPs) between any pair of parental plants (data from the parental flower bud transcriptomes). 'ma' refers to the read proportion of the minor allele (if any) used to define a SNP as (reciprocally) homozygous or heterozygous. The 'discriminant' base in the heterozygous case is depicted in red. (*B*) Schematic of our pipeline to infer maternal transcript proportions from both homozygous and heterozygous SNPs in the endosperm transcriptome data, and their analysis up to the designation of candidate imprinted genes. Candidate imprinted genes are identified as those exceeding our thresholds in *both directions* of the reciprocal cross. All maternal parents will be 'mosaics' of homozygous and heterozygous genotypes across heterozygous SNPs. See *Materials and Methods* for detailed explanations.

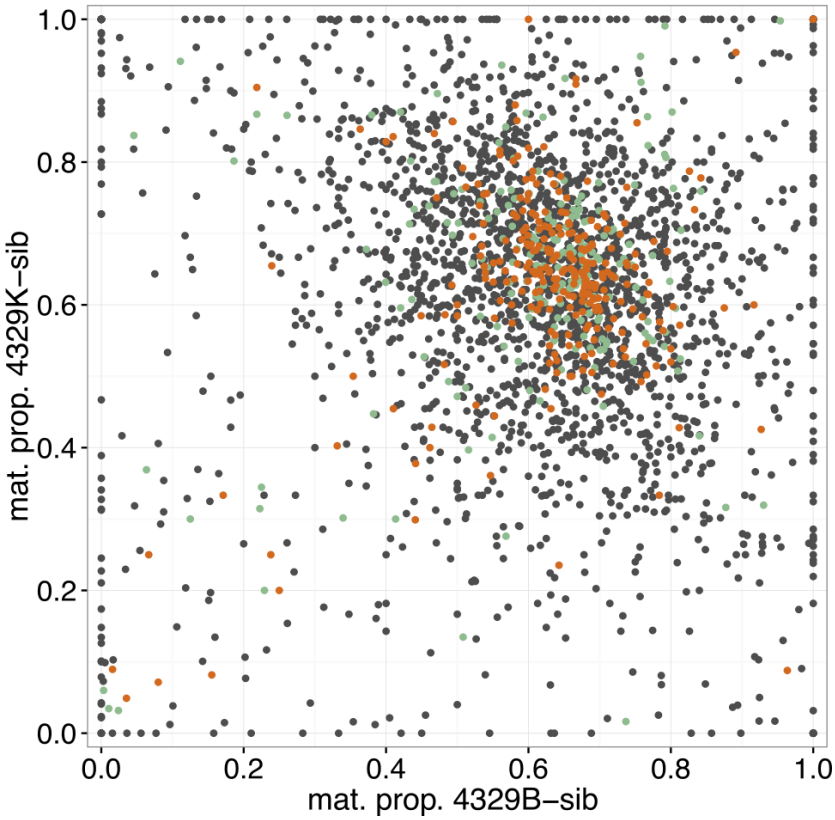

FIG. S2. Overview of *S. chilense* data from the reciprocal intraspecific crosses. Endosperm maternal proportions for 2,560 genes in the crosses 4329B × 4329K (*x* axis) and 4329K × 4329B (*y* axis). Candidate MEGs have maternal proportions >0.833 in both directions of the cross (upper right sector), and candidate PEGs have maternal proportions <0.333 in both directions of the cross (lower left sector). Three classes of genes are distinguished by color: ASE information from only heterozygous SNPs (gray dots, $n = 2{,}078$), ASE information from only homozygous SNPs (orange dots, $n = 278$), and ASE information from both types of SNPs (green dots, $n = 204$).

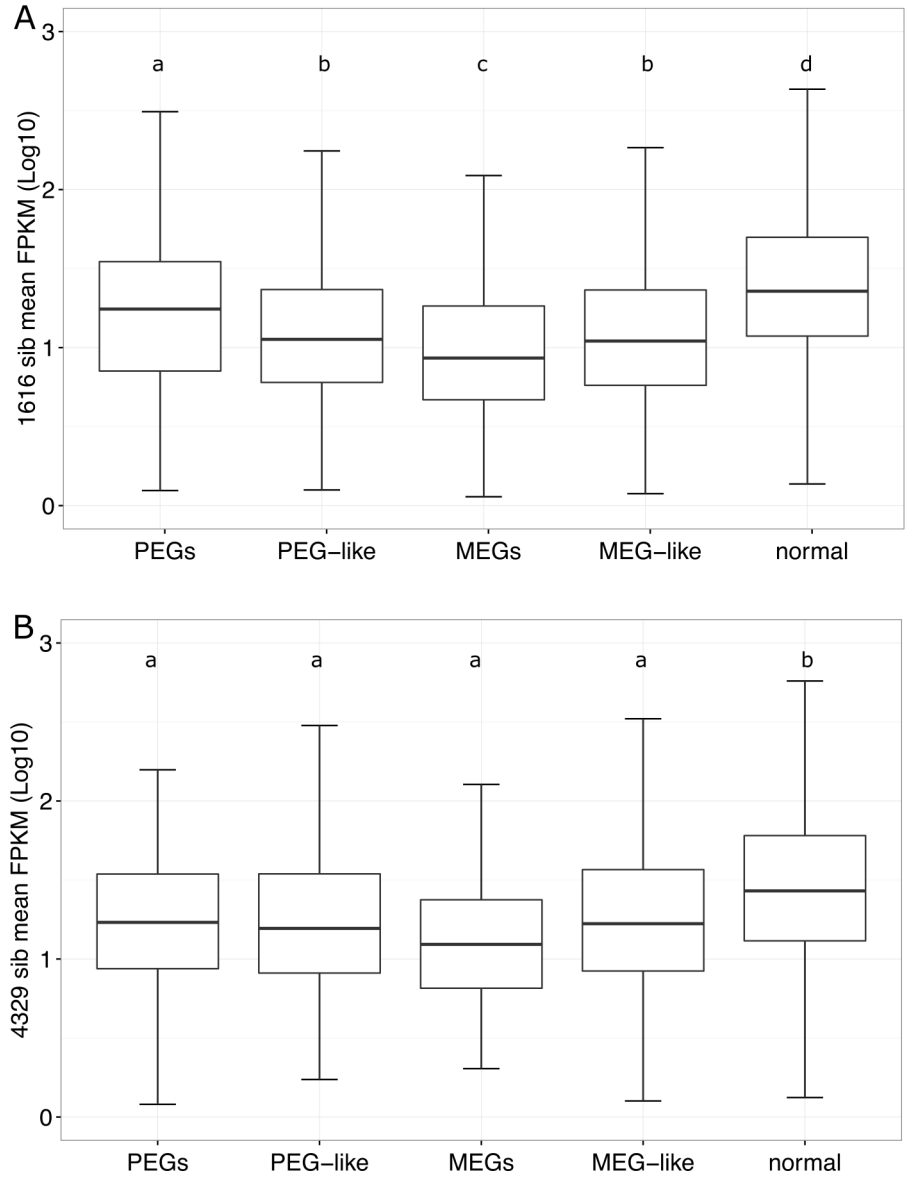

FIG. S3. Expression level of genes with uni- or bidirectional ASE is lower compared to those with 'normal' biallelic expression. (*A*) *S. peruvianum* candidate imprinted genes (PEGs and MEGs), genes in the same ranges of maternal proportion (i.e. <0.333 or >0.833) but only in one cross direction (735 'PEG-like' and 1,173 'MEG-like' genes), and genes with maternal proportions >0.333 and <0.833 in both cross directions (5,125 'normal' genes). (*B*) *S. chilense* candidate imprinted genes (PEGs and MEGs), 287 'PEG-like', 354 'MEG-like', and 1,641 'normal' genes. Expression level is plotted as (log10) FPKM, obtained by averaging (per gene) both within-population ('sib') crosses per species. Thick horizontal lines within box plots indicate the medians, and whiskers extend to 1.5× the interquartile range in both directions; outliers were excluded. Lower-case letters above the box plots indicate significance (pairwise Wilcoxon rank sum tests with Bonferroni *p*-value corrections).

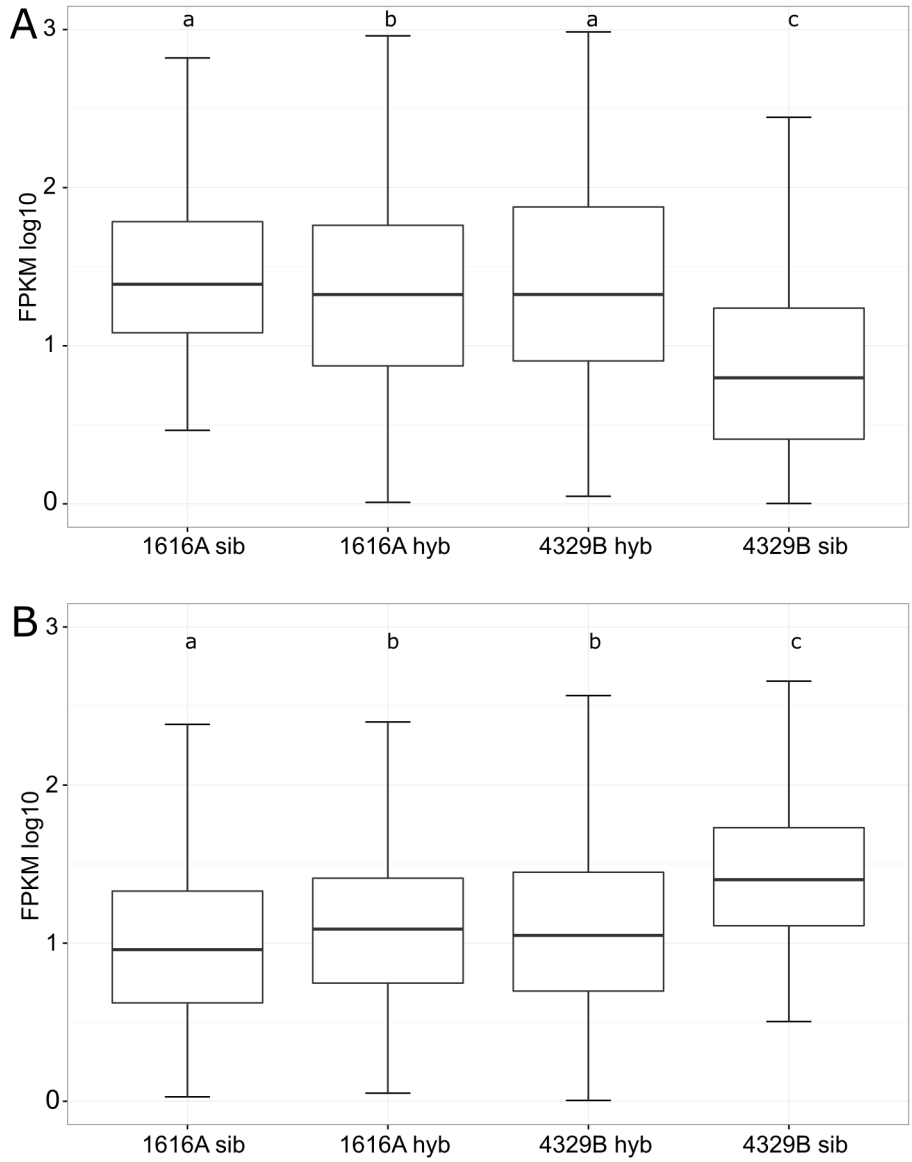

FIG. S4. Expression-level patterns of genes differentially expressed between *S. peruvianum* (1616A) and *S. chilense* (4329B), and comparisons with hybrid endosperm expression patterns on the same seed parents. (*A*) 867 genes upregulated in *S. peruvianum* compared to *S. chilense*, and (*B*) 647 genes upregulated in *S. chilense* compared to *S. peruvianum* (see *Materials and Methods* for details). Expression level is plotted as (log10) FPKM. Thick horizontal lines within box plots indicate the medians, and whiskers extend to 1.5× the interquartile range in both directions; outliers were excluded. Lower-case letters above the box plots indicate significance (pairwise Wilcoxon rank sum tests with Bonferroni *p*-value corrections).

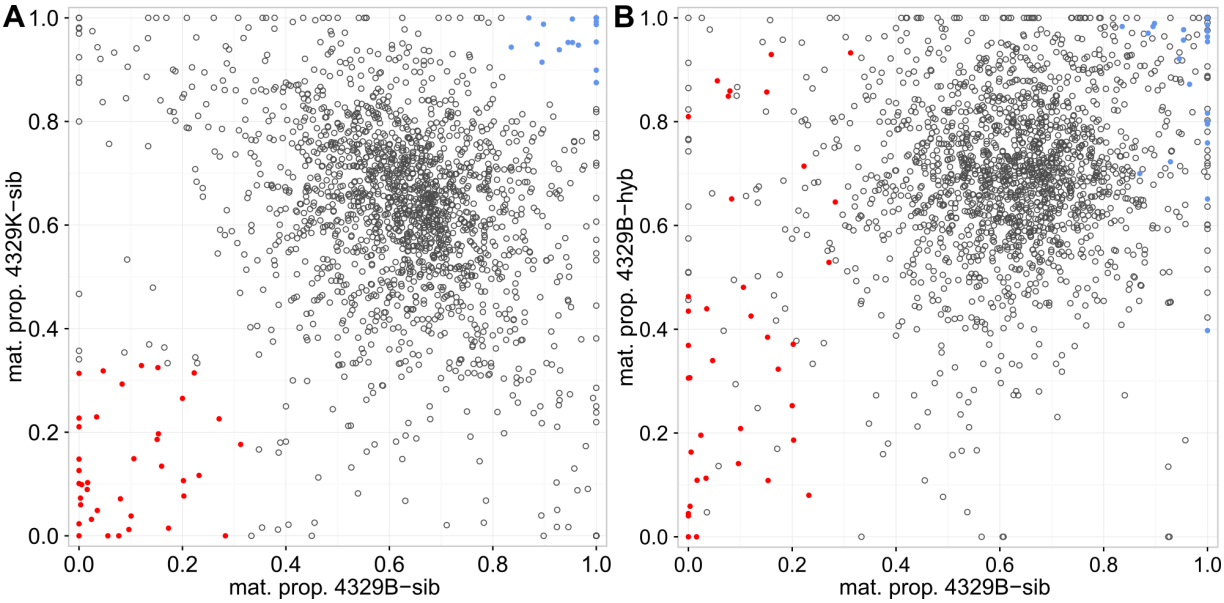

FIG. S5. Changes in maternal proportions for *S. chilense* genes with ASE information from both within-species and the independent hybrid crosses. (*A*) Endosperm maternal proportions for 1,856 genes in the reciprocal *S. chilense* crosses 4329B × 4329K (*x* axis) and 4329K × 4329B (*y* axis); the same cross as in fig. S2, but restricted to those genes with independent ASE information from the hybrid cross. Red and blue dots mark candidate PEGs and MEGs, respectively, and all other genes are marked as open circles. (*B*) Comparison of endosperm maternal proportions for the same 1,856 genes for 4329B in the within-species cross maternal role (*x* axis; same data as in *A*) *vs.* 4329B in the hybrid cross maternal role (*y* axis, 4329B × 1616A). Red and blue dots identify (within-species) candidate PEGs and MEGs, respectively.

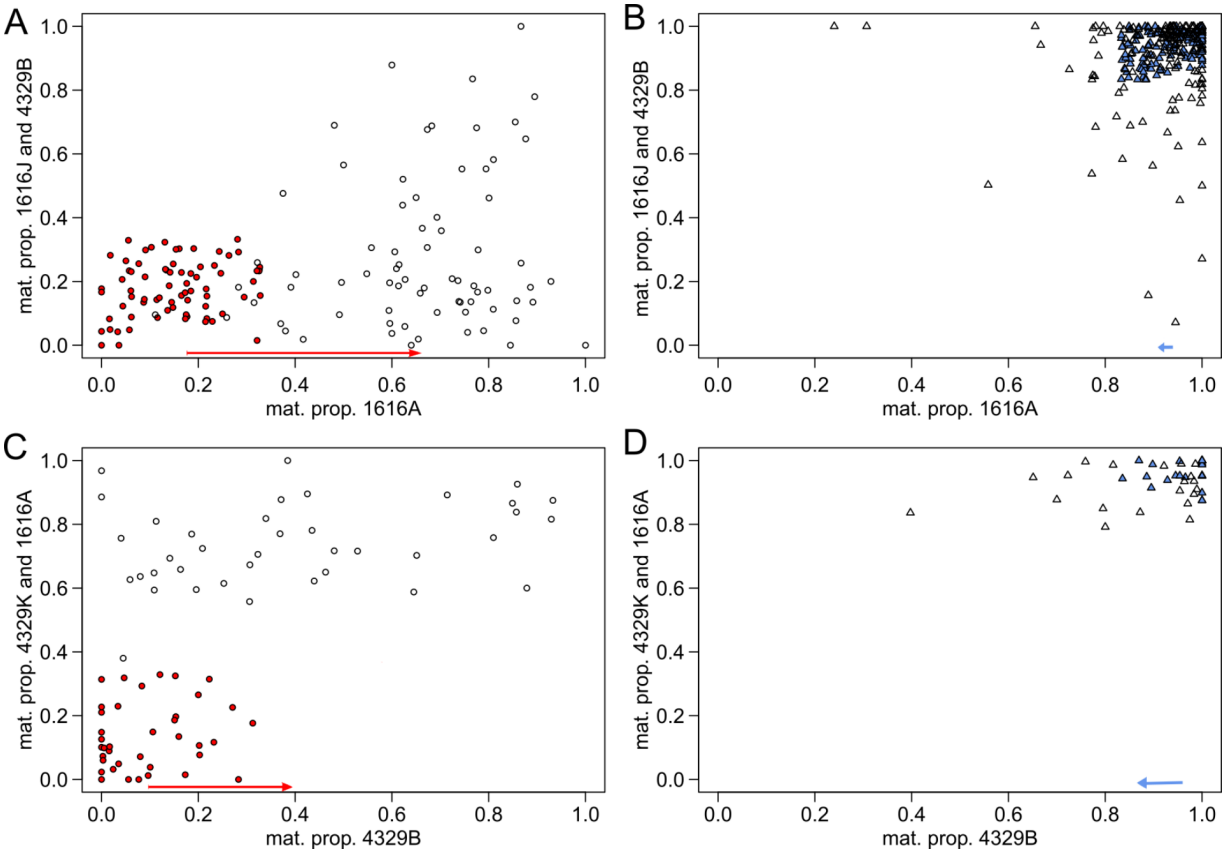

FIG. S6. Shift in maternal proportions of candidate PEGs and MEGs identified separately in both wild tomato species. (*A*) Shift in maternal proportion between within-population and hybrid endosperm for 73 candidate PEGs in *S. peruvianum* (LA1616). For within-population crosses (1616A ↔ 1616J), PEGs are indicated as red dots, and their respective maternal proportion in hybrid endosperm (1616A ↔ 4329B) is shown with open symbols. The red arrow along the *x* axis shows the average shift in maternal proportion from 1616A sib to hybrid endosperm. (*B*) Shift in maternal proportion between within-population and hybrid endosperm for 145 candidate MEGs in *S. peruvianum* (LA1616). For within-population crosses (1616A ↔ 1616J), MEGs are indicated as blue triangles, and their respective maternal proportion in hybrid endosperm (1616A ↔ 4329B) is shown with open symbols. The blue arrow along the *x* axis shows the average shift in maternal proportion from 1616A sib to hybrid endosperm. (*C*) Shift in maternal proportion between within-population and hybrid endosperm for 38 candidate PEGs in *S. chilense* (LA4329). For within-population crosses (4329B ↔ 4329K), PEGs are indicated as red dots, and their respective maternal proportion in hybrid endosperm (4329B ↔ 1616A) is shown with open symbols. The red arrow along the *x* axis shows the average shift in maternal proportion from 4329B sib to hybrid endosperm. (*D*) Shift in maternal proportion between within-population and hybrid endosperm for 21 candidate MEGs in *S. chilense* (LA4329). For within-population crosses (4329B ↔ 4329K), MEGs are indicated as blue triangles, and their respective maternal proportion in hybrid endosperm (4329B ↔ 1616A) is shown with open symbols. The blue arrow along the *x* axis shows the average shift in maternal proportion from 4329B sib to hybrid endosperm.

Table S1. Total Sequencing Output and Reads Mapped to the Reference Genome for Each of the Individual Illumina TruSeq Endosperm-Derived Libraries.

Library identifier	raw number of reads (million)	uniquely mapped reads (million)
LA1616A_hyb_endos_rep1	18.19	14.77
LA1616A_hyb_endos_rep2	14.87	12.33
LA1616A_sib_endos_rep1	14.60	12.41
LA1616A_sib_endos_rep2	35.80	30.54
LA1616J_sib_endos_rep1	39.10	33.08
LA1616J_sib_endos_rep2	35.30	29.35
LA4329B_hyb_endos_rep1	12.24	9.93
LA4329B_hyb_endos_rep2	17.65	14.54
LA4329B_sib_endos_rep1	35.61	30.11
LA4329B_sib_endos_rep2	18.20	14.75
LA4329K_sib_endos_rep1	18.26	14.72
LA4329K_sib_endos_rep2	29.51	24.85

Table S2. Summary Information for All Candidate Imprinted Genes in *Solanum peruvianum* and *Solanum chilense*. (separate **Excel file**)

NOTE.—Gene names and functional annotation are given based on the ITAG Release 2.3 of the tomato reference genome sequence. Maternal proportions for both directions of reciprocal crosses are given, and based on these the designated category of imprinting ('moderate', 'strong' or 'complete'). Also included are the total number of SNPs (per gene) used for the estimation of maternal transcript proportions (column F), broken down into the number of hetero- (column G) and homozygous (column H) parental differences. Colored entries mark candidate imprinted genes shared between the two species (column A, pink), genes encoding proteins with functions in the chloroplast (green, column K), and putative SCF protein-complex genes (blue, column K). Finally, *p*-values for χ^2 tests (column I) and False Discovery Rate (FDR) corrections (column J) are also listed.

Table S3. Summary of Possible Outcomes Under Mis-Specification of Parental Genotypes Due to Potential Allele-Specific Expression (ASE) in the Flower Bud Transcriptome of Parental Plants.

True genotypes	Scored genotypes	Logic/Comments	Endosperm expression →	Endosperm expectations →	Inference on mat. prop.	Conclusions
Case 1: 'apparent' homozygote × true homozygote						
AT	AA	any T counted as paternal	normal 2:1	1/3 A, 2/3 T	0.333	No spurious candidate
×	×		ASE like in buds	2/3 A, 1/3 T	0.666	MEGs/PEGs expected; rather,
TT	TT	any A counted as paternal	normal 2:1	1/6 A, 5/6 T	0.833	apparent plant-specific expression
			ASE like in buds	1/3 A, 2/3 T	0.666	possible
Case 2: 'apparent' homozygote × true heterozygote						
AT	AA		normal 2:1	1/2 A, 1/2 T	0.000	No spurious candidate MEGs/
×	×	T will be scored as	ASE like in buds	5/6 A, 1/6 T	0.666	PEGs expected; rather, "normal"
AT	AT	"discriminant" allele	normal 2:1	1/2 A, 1/2 T	1.000	endosperm expression causes
			ASE like in buds	2/3 A, 1/3 T	0.666	apparent plant-specific expression
Case 3: 'apparent' homozygote × 'apparent' homozygote						
AT	AA	any T counted as paternal	normal 2:1	1/2 A, 1/2 T	0.500	No spurious candidate
×	×		ASE like in buds	2/3 A, 1/3 T	0.666	MEGs/PEGs expected
AT	TT	any A counted as paternal	normal 2:1	1/2 A, 1/2 T	0.500	
			ASE like in buds	1/3 A, 2/3 T	0.666	

NOTE.—'Apparent' homozygote refers to heterozygous genotypes mis-scored as homozygotes due to ASE. There are three possible combinations of informative parental genotypes (cases 1–3, always considering reciprocal crossings). Consequences of divergent endosperm expression of alleles derived from apparent homozygotes as both fathers and mothers are considered, either 'normal 2:1' ratio or 'ASE like in buds' (i.e. the latter case preserving the bud-tissue ASE in the endosperm). For each case, the inferred maternal proportions are highlighted with the same colors for reciprocal crosses (orange, normal 2:1; light blue, ASE like in buds). Mis-scoring of heterozygotes as homozygotes is not expected to generate spurious candidate imprinted genes. To the contrary, two of the three cases might yield loci with apparent plant-specific expression, for which there is little evidence in our data (figs. 2, 3).