SUPPLEMENTAL MATERIAL

The Evolution of Inflorescence Diversity in the Nightshades and Heterochrony during Meristem Maturation

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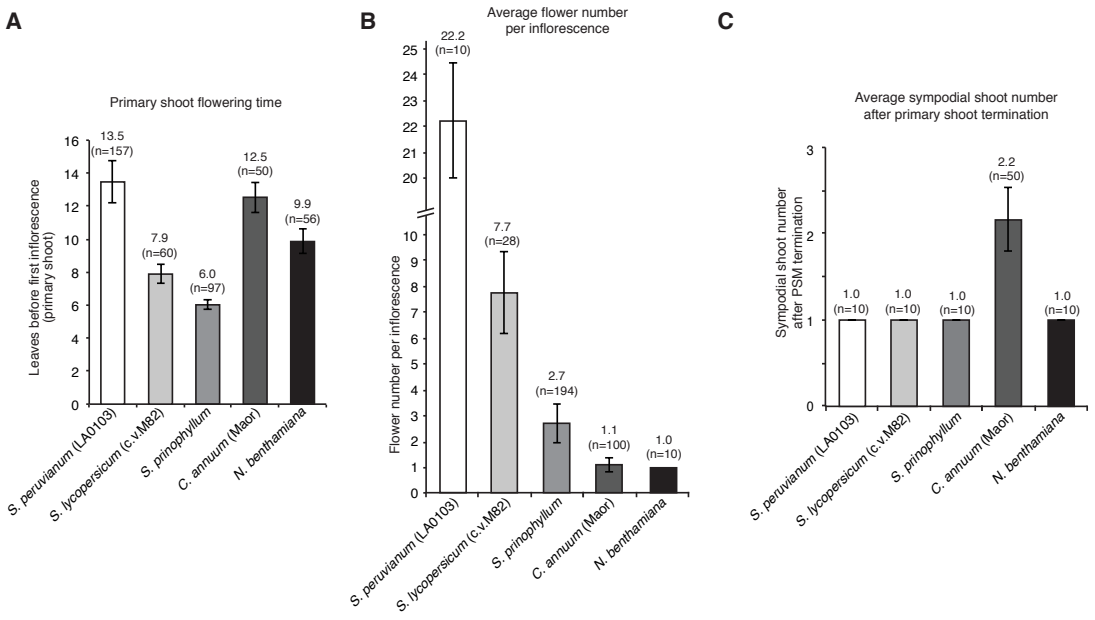
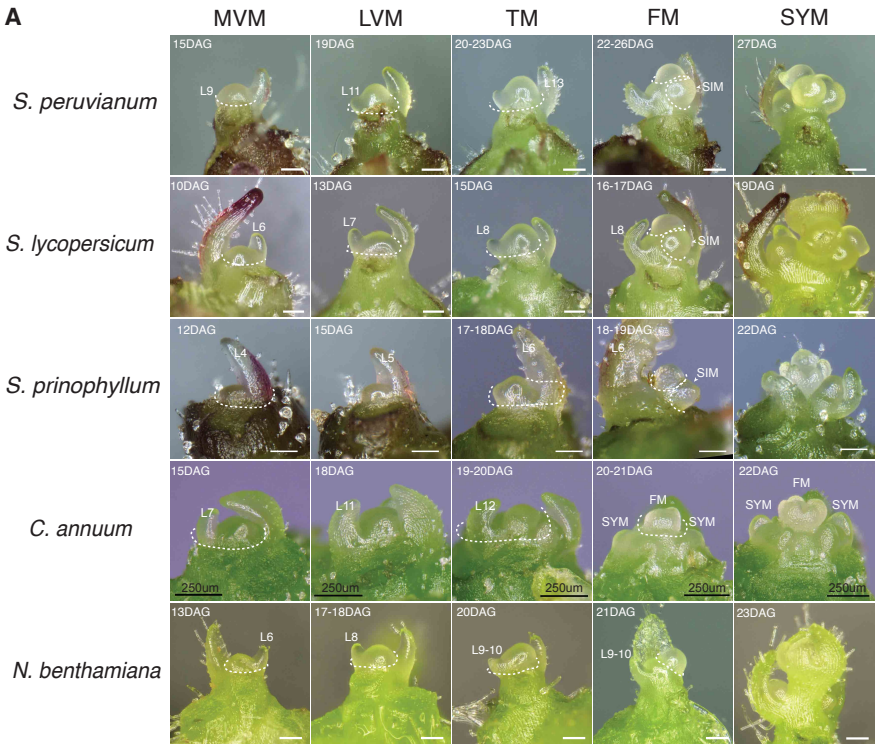
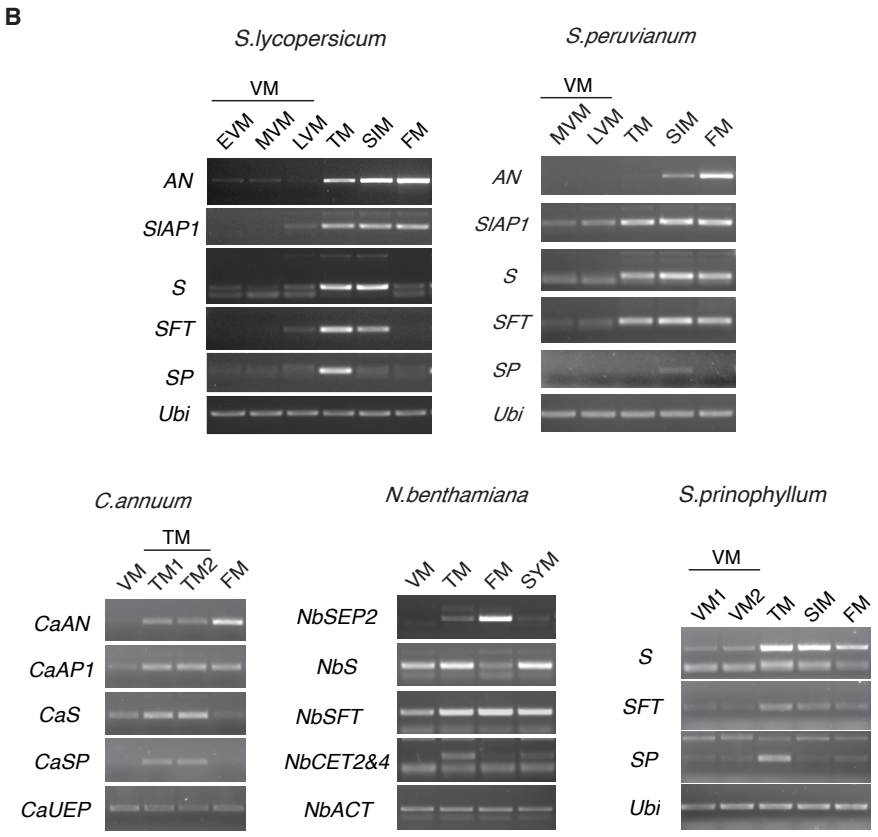
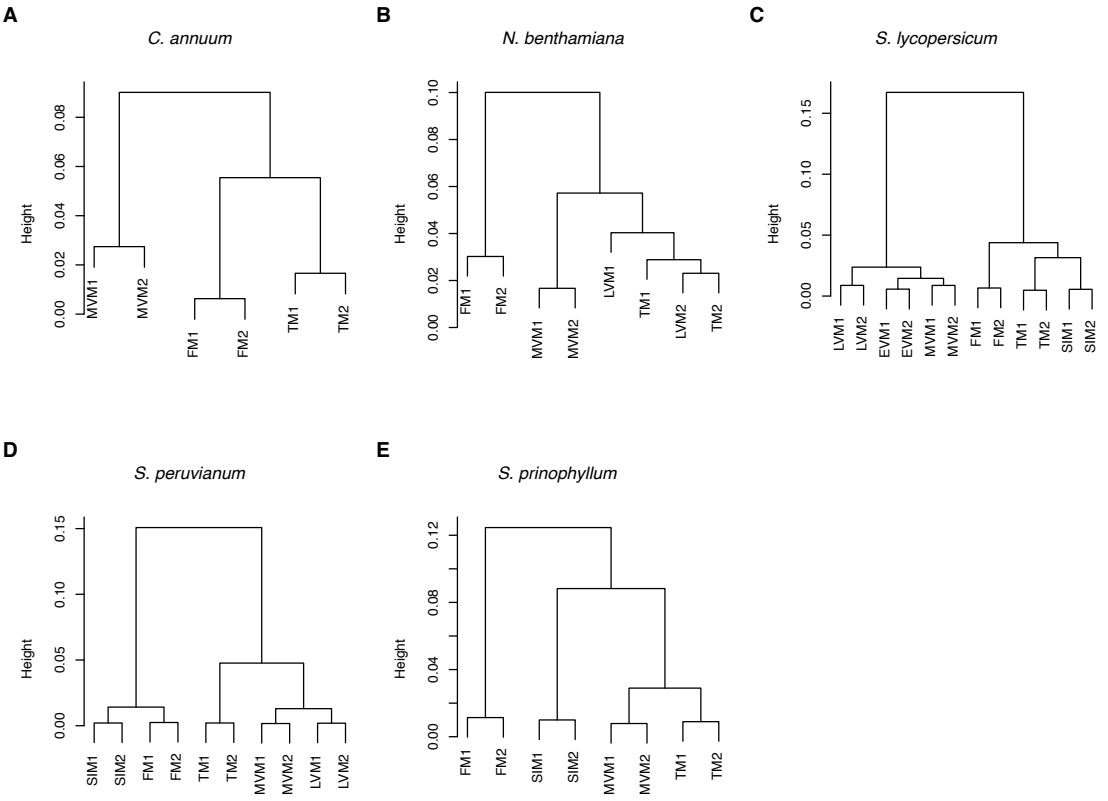


Supplemental Figure S1. Flowering time, flower production, and sympodial index summary from five *Solanaceae* species. The above flowering related traits were quantified in *S. peruvianum*, *S. lycopersicum*, *S. prinophyllum*, *C. annuum*, and *N. benthamiana.* (*A*) Flowering time of the primary shoot in leaf number. (*B*) Average flower number per inflorescence. (*C*) Average sympodial shoot number after primary shoot termination.

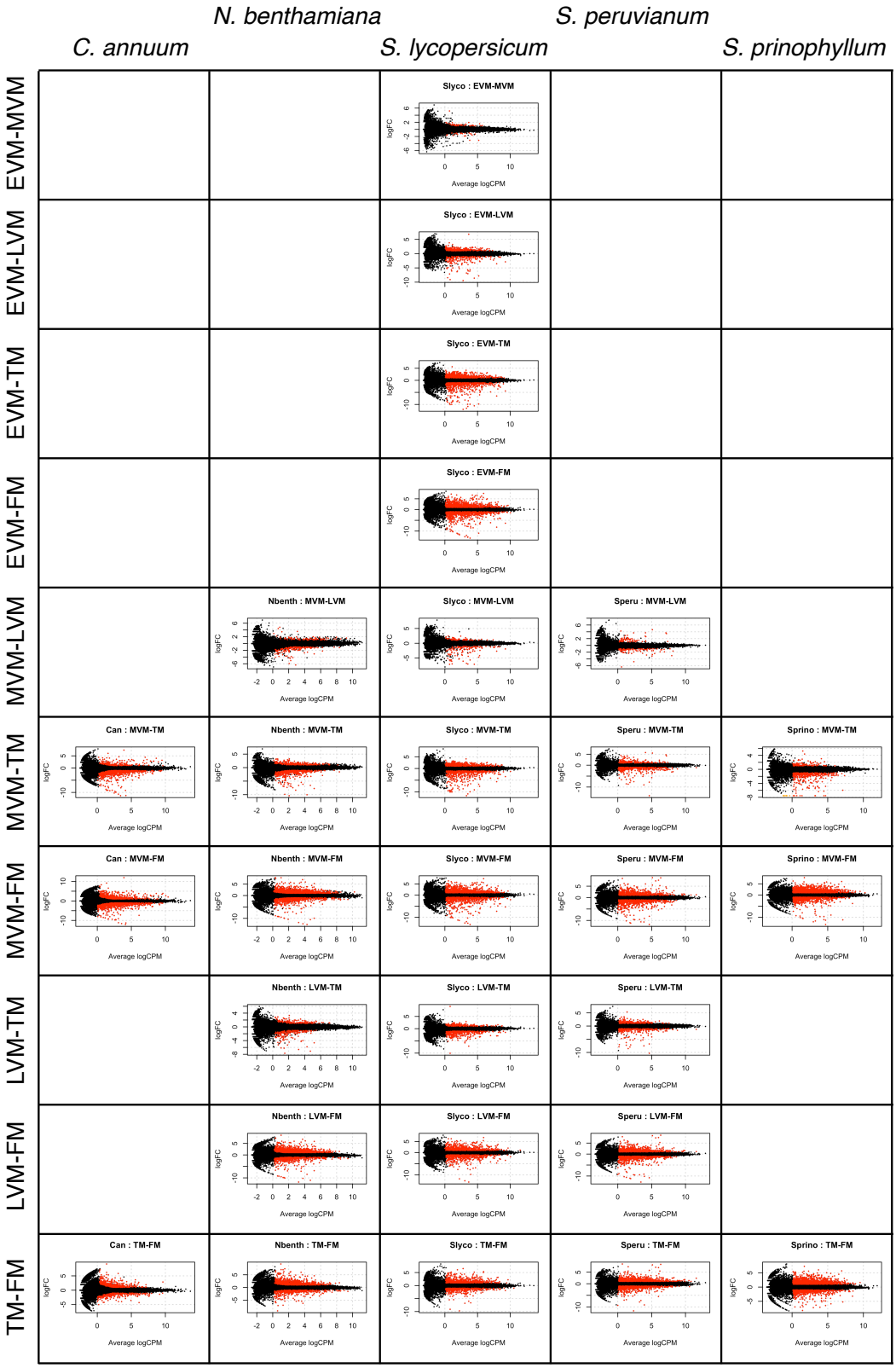

A
MVM
LVM
TM
FM
SYM
S. peruvianum
S. lycopersicum
S. prinophyllum
C. annuum
N. benthamiana
15DAG
19DAG
20-23DAG
22-26DAG
27DAG
10DAG
13DAG
15DAG
16-17DAG
19DAG
12DAG
15DAG
17-18DAG
18-19DAG
22DAG
15DAG
18DAG
19-20DAG
20-21DAG
22DAG
13DAG
17-18DAG
20DAG
21DAG
23DAG
L9
L11
L13
SIM
L6
L7
L8
L4
L5
L12
FM
SYM
L9-10
250um


B
S.lycopersicum
S.peruvianum
VM
EVM
MVM
LVM
TM
SIM
FM
AN
SlAP1
S
SFT
SP
Ubi
C.annuum
N.benthamiana
S.prinophyllum
CaAN
CaAP1
CaS
CaSP
CaUEP
TM1
TM2
NbSEP2
NbS
NbSFT
NbCET2&4
NbACT
SYM
VM1
VM2

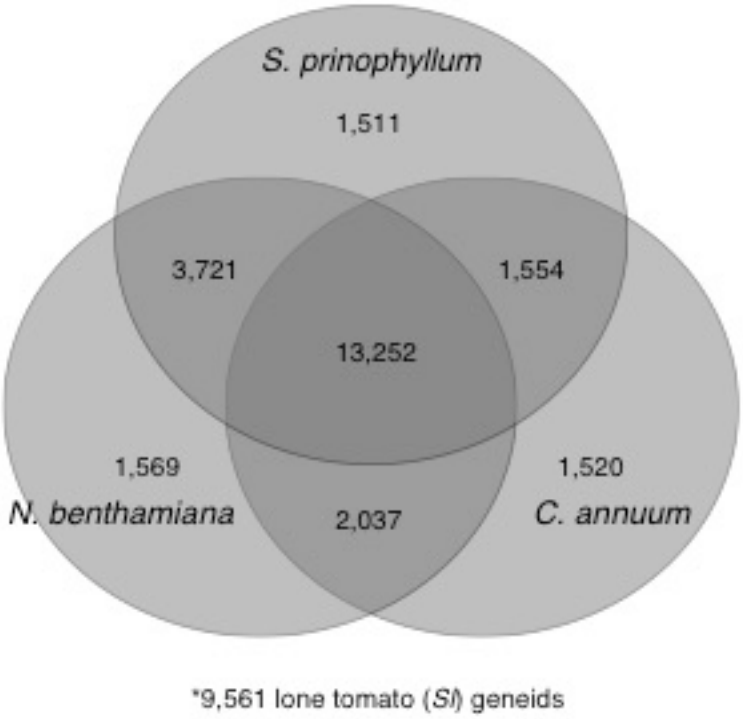
Supplemental Figure S2. (*A*) Expanded analysis of meristem ontogeny. Developmental stages of the primary shoot meristem include: *E*arly *V*egetative *M*eristem (EVM), *M*iddle *V*egetative *M*eristem (MVM), *L*ate *V*egetative *M*eristem (LVM), *T*ransition *M*eristem (TM), and *F*loral *M*eristem (FM) with associated *S*ympodial *I*nflorescence *M*eristem (SIM). The meristem that gives rise to the *SY*mpodial shoot *M*eristem (SYM) is located in the axil of the last initiated leaf and gives rise to additional vegetative growth and terminal floral meristems. White scale bars represent 100 μm. Reference images of meristem stages for all species except *S. prinohyllum* and *C. annuum* were adapted from (MacAlister et al. 2012; Park et al. 2012). (*B*) Semi-quantitative RT-PCR using meristem stage specific marker genes for validation of all stages among species. *SELF PRUNING* (*SP*) orthologs (*CaSP* and *NbCET2&4*) were used as markers for the TM and SYM; *SINGLE FLOWER TRUSS* (*SFT*) orthologs (*NbSFT*) were used as markers for transition VM to TM; *APETALA1* or *SEPALLATA2* orthologs (*CaAP1* and *NbSEP2*) were used as markers for TM, SIM, and FM; *S* and *AN* orthologs (*CaS*, *NbS*, *CaAN* and *NbAN*) were sequentially enriched in the SIM and FM stages, respectively (Park el al. 2012); *UBIQUITIN* (*S. lycopersicum*, *S. peruvianum* and *S. prinophyllum*), *CaUEP* (*C. annuum Ubiquitin Extension Protein*), *NbACT* (*N. benthamiana Actin*) was used as a reference.



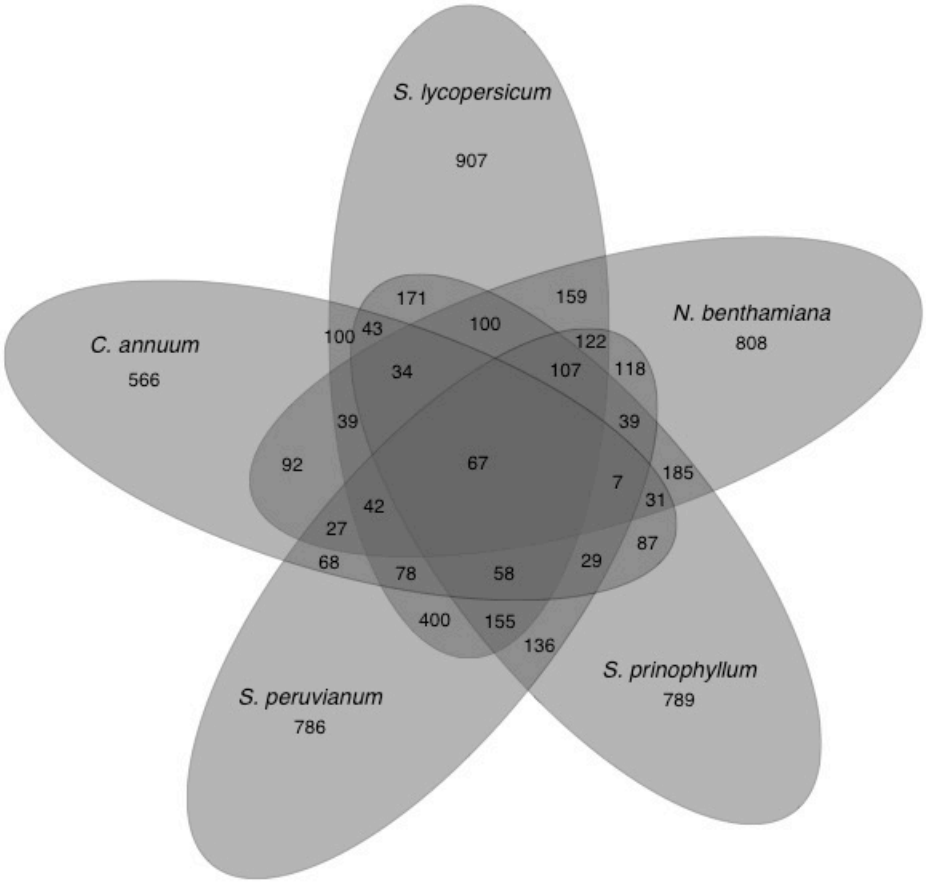
Supplemental Figure S3. Transcriptome dendrograms of expressed genes. Euclidian distance dendrograms for each replicate of EVM, MVM, LVM, TM, and FM for the five species for genes with sum expression greater than 20 fragments.


N. benthamiana
S. peruvianum
C. annuum
S. lycopersicum
S. prinophyllum
EVM-MVM
EVM-LVM
EVM-TM
EVM-FM
MVM-LVM
MVM-TM
MVM-FM
LVM-TM
LVM-FM
TM-FM
Slyco : EVM-MVM
Slyco : EVM-LVM
Slyco : EVM-TM
Slyco : EVM-FM
Nbenth : MVM-LVM
Slyco : MVM-LVM
Speru : MVM-LVM
Can : MVM-TM
Nbenth : MVM-TM
Slyco : MVM-TM
Speru : MVM-TM
Sprino : MVM-TM
Can : MVM-FM
Nbenth : MVM-FM
Slyco : MVM-FM
Speru : MVM-FM
Sprino : MVM-FM
Nbenth : LVM-TM
Slyco : LVM-TM
Speru : LVM-TM
Nbenth : LVM-FM
Slyco : LVM-FM
Speru : LVM-FM
Can : TM-FM
Nbenth : TM-FM
Slyco : TM-FM
Speru : TM-FM
Sprino : TM-FM
logFC
Average logCPM

Supplemental Figure S4. MA plots for each pairwise stage contrast by species. Red dots indicate significant differential expression as determined by edgeR with average counts per million ≥ 1.0, minimum of 2- foldchange, and FDR ≤ 0.10. Species included are *Capsicum annuum*, *Nicotiana benthamiana*, *S. lycopersicum*, *S. peruvianum*, and *S. prinophyllum*.



Supplemental Figure S5. Number of orthogroups with members in each species. A total of 9,561 orthogroups have members from all four species. Approximately 4,500 additional orthogroups are missing a member from one species, while ~7,200 are missing members from two species. From the reference tomato gene set (34,725 genes) 13,252 have zero identified ortholog in the other species.



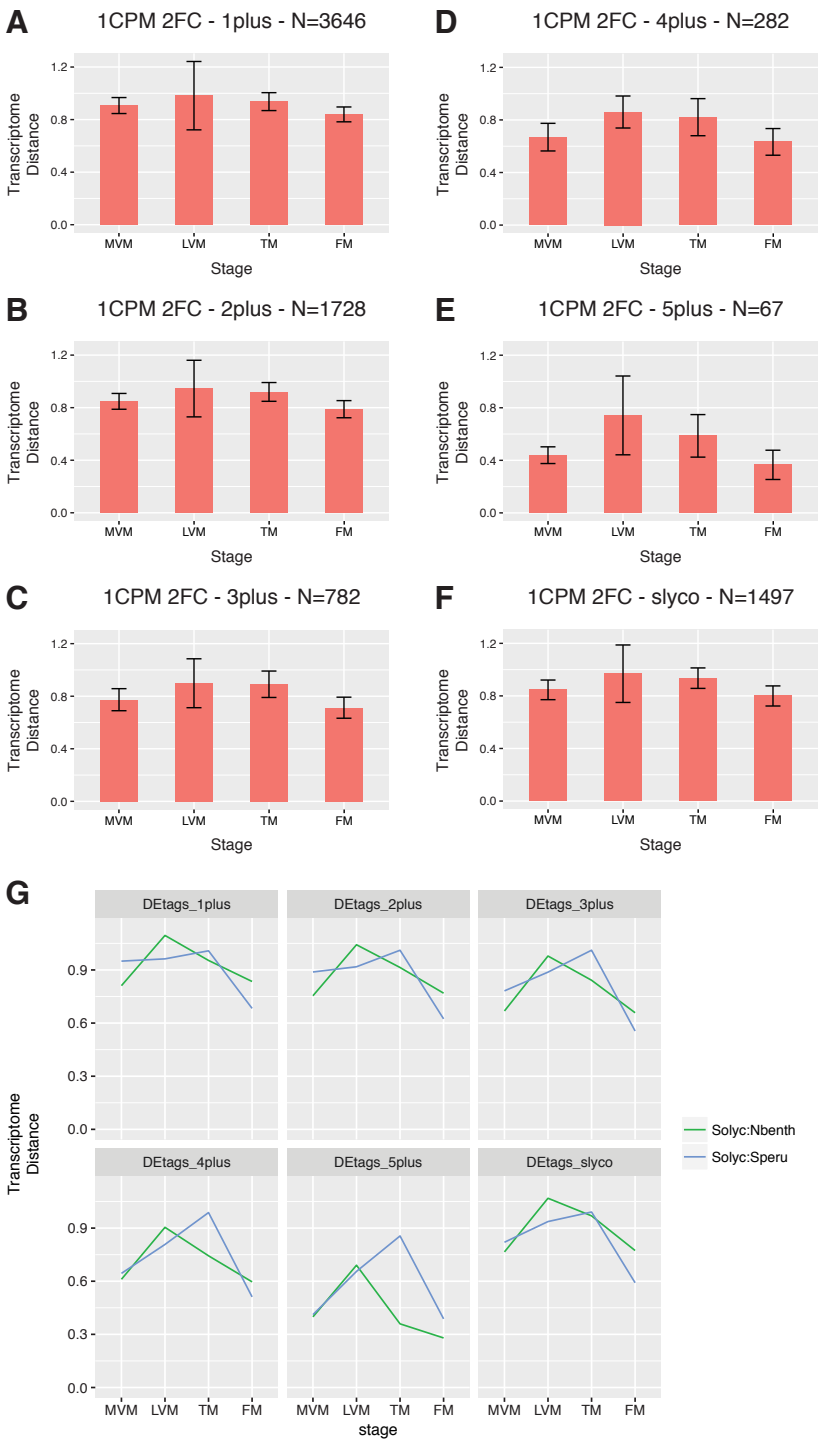
Supplemental Figure S6. Overlap of differentially expressed (DE) orthogroups between species. The overlap of orthogroups with DE members (determined by edgeR) between the five experimental species were counted and shown as a Venn diagram. There were a total of 3646, 1728, 782, 282, and 67 orthogroups with DE in one, two, three, four, and five species, respectively. Of these DE orthogroups, 1497 were DE in *S. lycopersicum*.

See supplemental file “Supplemental_Fig_S7.pdf”

Supplemental Figure S7. Clustering of z-score normalized transcriptional profiles for different subsets of differentially expressed orthogroups. Gene subsets include those identified as DE in at least (*A*) one, (*B*) two, (*C*) three, (*D*) four, and (*E*) five species. An additional set of genes DE in *S. lycopersicum* is also shown (*F*). The number of orthogroups (N) is indicated in the title of each heatmap, with high and low expression depicted by yellow and blue colors, respectively. Transcriptional profiles are ordered from left to right as follows: all genes with biological replicates combined, all genes with biological replicates separate, transcription factors with biological replicates combined, and transcription factors with biological replicates separate.

See supplemental file “Supplemental_Fig_S8.pdf”

Supplemental Figure S8. Principal component analysis (PCA) plots for different subsets of differentially expressed orthogroups. Gene subsets include those identified as DE in at least (*A*) one, (*B*) two, (*C*) three, (*D*) four, and (*E*) five species. An additional set of genes DE in *S. lycopersicum* is also shown (*F*). The number of orthogroups (N) included in the analysis is indicated in the plot area. The first two principal components are presented with shapes representing different species and colors the meristem maturation stage. PCA plots are ordered from left to right as follows: all genes with biological replicates combined, all genes with biological replicates separate, transcription factors with biological replicates combined, and transcription factors with biological replicates separate.

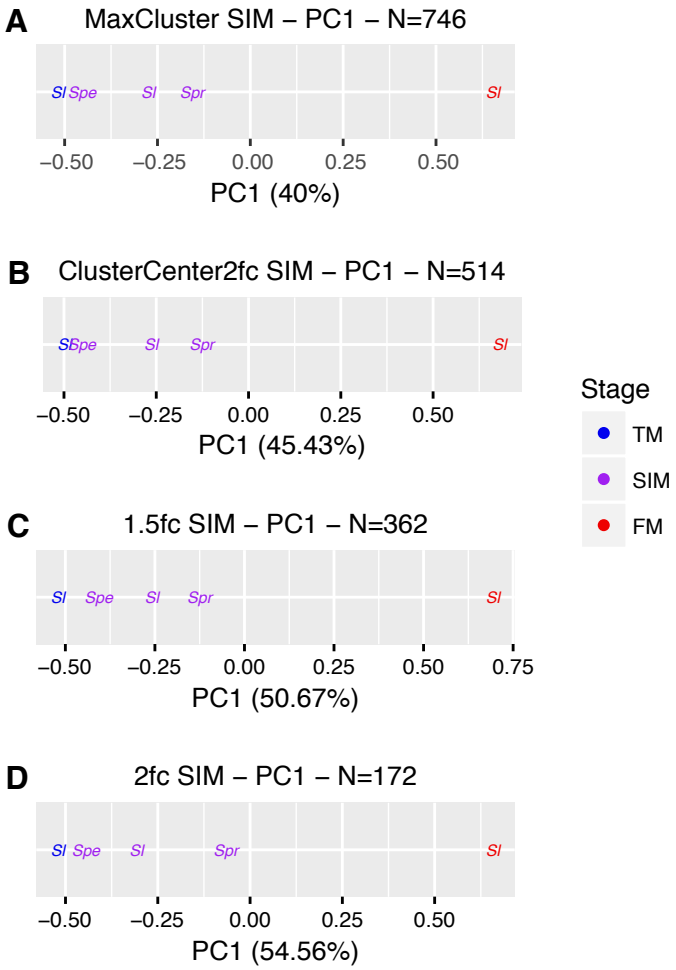


Supplemental Figure S9. Transcriptome expression divergence for different subsets of differentially expressed orthogroups. Gene subsets include those identified as DE in at least (*A*) one, (*B*) two, (*C*) three, (*D*) four, and (*E*) five species. An additional set of genes DE in *S. lycopersicum* is also shown (*F*). The number of orthogroups (N) used in

calculating transcriptome distance is indicated in the title of each bar graph. Transcriptome distance peaks during the LVM and TM of all gene sets, indicating a peak of transcriptome expression divergence occurs during a transitional window of meristem maturation. (*G*) Transcriptome expression divergence at the various cutoffs for *S. lycopersicum* versus *N. benthamiana* (Solyc:Nbenth; green line) and *S. lycopersicum* versus *S. peruvianum* (Solyc:Speru; blue line). Notably, the *S. lycopersicum* contrast with *N. benthamiana* is always highest in the LVM and with *S. peruvianum* always highest in the TM.

See supplemental file “Supplemental_Fig_S10.pdf”

Supplemental Figure S10. Full *k*-means tomato clusters and other species’ *k*-means subclusters. Gene expression from tomato was clustered using 12 *k*-means clusters. Expression from other species orthologous genes was then sub-clustered for each original tomato cluster (10 sub-clusters per tomato cluster). Vertical cluster numbers refer to tomato clusters and horizontal cluster numbers refer to other species subclusters. The number of genes (N) in each specific Cluster:Subcluster (C:S) is also indicated above. Sub-clustering was performed separately for each species in the study: (*A*) *N. benthamiana*, (*B*) *C. annuum*, (*C*) *S. prinophyllum*, and (*D*) *S. peruvianum*.



Supplemental Figure S11. Sympodial Inflorescence Meristem (SIM) principal component analysis (PCA). Gene expression of several TM/FM marker gene sets was subjected to PCA for *S. lycopersicum* (*Sl*) TM, SIM, and FM in addition to SIM of *S. peruvianum* (*Spe*) and *S. prinophyllum* (*Spr*). (*A*) MaxCluster – all genes from the *k*-means clusters with max expression in the TM or FM. (*B*) ClusterCenter2fc – all genes from the *k*-means clusters where the center for the TM or FM was 2-fold greater than all other stage centers. (*C*) 1.5fc – all genes expressed 1.5-fold higher in the TM or FM on a gene-by-gene basis. (*D*) 2fc – all genes expressed 2-fold higher in the TM or FM on a gene-by-gene basis (gene set used in Figure 4).

See supplemental file “Supplemental_Fig_S12.pdf”

Supplemental Figure S12. *COMPOUND INFLORESCENCE* (*S*) and *ANANTHA* (*AN*) *in situ* hybridization serial sections for *S. lycopersicum* (*Sl*). Serial sections for several apices are shown at 10x magnification. Both *S* (*A*) and *AN* (*B*) individuals are ordered by approximate age with the youngest individuals to the left. Images used in Figure 5 are marked with a red square. Black scale bars represent 100 μm.

See supplemental file “Supplemental_Fig_S13.pdf”

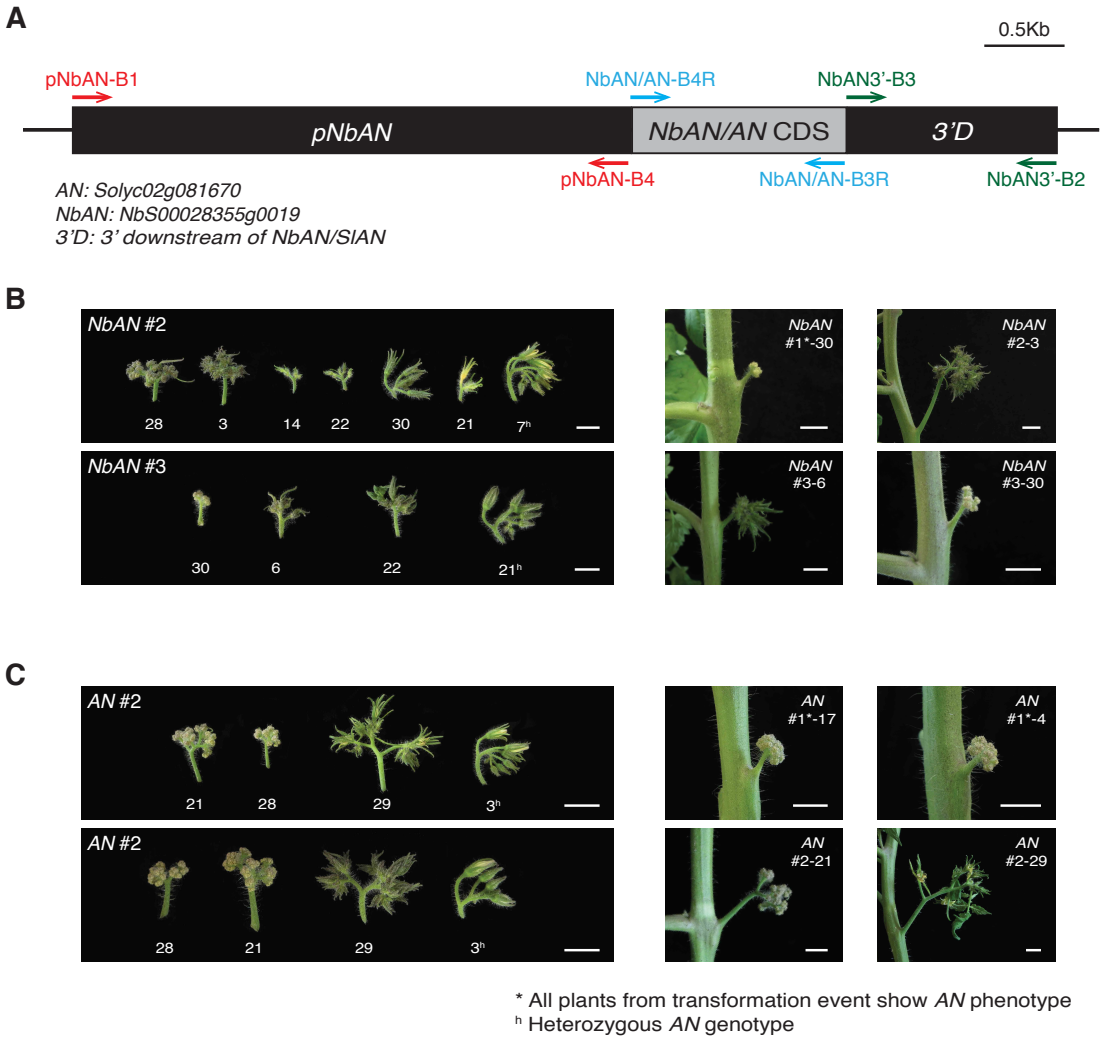
Supplemental Figure S13. *COMPOUND INFLORESCENCE* (*S*) and *ANANTHA* (*AN*) *in situ* hybridization serial sections for *N. benthamiana* (*Nb*). Serial sections for several apices are shown at 10x magnification. Both *S* (*A*) and *AN* (*B*) individuals are ordered by approximate age with the youngest individuals to the left. Images used in Figure 5 are marked with a red square. Black scale bars represent 100 μm.

See supplemental file “Supplemental_Fig_S14.pdf”

Supplemental Figure S14. *COMPOUND INFLORESCENCE* (*S*) and *ANANTHA* (*AN*) *in situ* hybridization serial sections for *S. peruvianum* (*Spe*). Serial sections for several apices are shown at 10x magnification. Both *S* (*A*) and *AN* (*B*) individuals are ordered by approximate age with the youngest individuals to the left. Images used in Figure 5 are marked with a red square. Black scale bars represent 100 μm.

See supplemental file “Supplemental_Fig_S15.pdf”

Supplemental Figure S15. *COMPOUND INFLORESCENCE* (*S*) and *ANANTHA* (*AN*) *in situ* hybridization serial sections for *S. prinophyllum* (*Spr*). Serial sections for several apices are shown at 10x and 20x magnification. Both *S* (*A*) and *AN* (*B*) individuals are ordered by approximate age with the youngest individuals to the left. Images used in Figure 5 are marked with a red square. Black scale bars represent 100 μm.



Supplemental Figure S16. *AN* cross species transgenic complementation test. Two transgenic constructs for expression of the tomato and tobacco AN coding sequence under tobacco regulatory sequences were constructed. (*A*) Tobacco regulatory sequences (3,475 basepairs upstream and 1,303 basepairs downstream) were fused with the tobacco and tomato *AN* coding sequence and transformed into a segregating tomato *an* mutant background. A variable degree of rescue was observed for both the tobacco (*B*) and tomato (*C*) constructs, suggesting simple expression of AN protein (tomato or tobacco) under the tobacco promoter sequence is insufficient to restore proper floral identity and additional factors are required. White scale bars represent 1 cm.

Supplemental Table S1. List of *Solanaceae* species surveyed for inflorescence diversity.

See supplemental file "Supplemental_Table_S1.xlsx"

Supplemental Table S2. Flowering time of experimental species.

Genotype	5 leaves	6 leaves	7 leaves	8 leaves	9 leaves	10 leaves	11 leaves	12 leaves	13 leaves	14 leaves	15 leaves	16 leaves	17 leaves	Averages of leaf no. (SD, N)
S. lycopersicum (M82)	0	0	21.7%	68.3%	10.0%	0	0	0	0	0	0	0	0	7.9 (±0.56, 60)
S. peruvianum (LA0103)	0	0	0	0	0	0.6%	6.4%	13.4%	28.0%	33.8%	13.4%	3.2%	1.3%	13.5 (±1.26, 157)
S. prinophyllum	3.1%	91.8%	5.2%	0	0	0	0	0	0	0	0	0	0	6.0 (±0.29, 97)
C. annuum (Maor)	0	0	0	0	0	0	8.0%	48.0%	30.0%	10.0%	4.0%	0	0	12.5 (±0.93, 50)
N. benthamiana	0	0	0	0	33.9%	48.2%	16.1%	1.8%	0	0	0	0	0	9.9 (±0.75, 56)

SD, standard deviation; N, number of total plants

Supplemental Table S3. Information on RNA sequencing reads such as sequencing platform, library kit used, total number of reads, number of mapped reads, and proportion of mapped reads.

See supplemental file "Supplemental_Table_S3.xlsx"

Supplemental Table S4. Number of reference genes from tomato, potato, and pepper reconstructed by the *S. prinophyllum de novo* assembly.

Proportion of reference reconstructed	Tomato	Potato	Pepper
0-10%	83	110	91
10-20%	444	512	386
20-30%	603	643	607
30-40%	665	746	679
40-50%	821	869	832
50-60%	1129	1161	1053
60-70%	1137	1202	1127
70-80%	1236	1234	1306
80-90%	1525	1642	1747
90-100%	12609	12590	11340

Supplemental Table S5. Fragment (paired reads) counts associated with transcripts from the various species.

See supplemental file “Supplemental_Table_S5.xlsx”

Supplemental Table S6. Fragment (paired reads) counts per million associated with transcripts from the various species.

See supplemental file “Supplemental_Table_S6.xlsx”

Supplemental Table S7. Significantly differentially expressed (DE) genes determined by edgeR (2- foldchange, average 1 count per million, FDR 0.10) for the five species.

See supplemental file “Supplemental_Table_S7.xlsx”

Supplemental Table S8. Geneids from tomato, tobacco, pepper, and *S. prinophyllum* in each orthologous gene group (named after the tomato geneid) determined by pairwise OMA analysis.

See supplemental file "Supplemental_Table_S8.txt"

Supplemental Table S9. Precocious gene overlap counts between tobacco and pepper for several counts per million (CPM) and fold change (FC) filters.

See supplemental file "Supplemental_Table_S9.xlsx"

Supplemental Table S10. Precocious gene overlap orthogroups (given as tomato geneids) for several counts per million (CPM) and fold change (FC) cutoffs.

See supplemental file “Supplemental_Table_S10.xlsx”

Supplemental Table S11. Enriched Molecular Function GOslim terms in precocious transition and floral genes.

Description	Number in Query N = 74	Number in Background N = 9036	FDR
transcription factor activity	14 (18.9%)	375 (4.2%)	8.70×10^{-5}
DNA binding	18 (24.3%)	704 (7.8%)	4.30×10^{-4}
transcription regulator activity	14 (18.9%)	501 (5.5%)	6.00×10^{-4}
nucleic acid binding	18 (24.3%)	1130 (12.5%)	0.029

Supplemental Table S12. Primer sequences used in this study.

See supplemental file “Supplemental_Table_S12.xlsx”

SUPPLEMENTAL METHODS

Plant materials and growth conditions

For meristem collection, seeds from our five experimental species (*S. lycopersicum*, *S. peruvianum*, *S. prinophyllum*, *C. annuum*, and *N. benthamiana*) were pre-germinated on fully wet Whatman paper in petri dishes at 28°C. After germination, seedlings at a similar stage were sown in 72-cell flats under natural light in a greenhouse supplemented with artificial light from high-pressure sodium bulbs (16h/8h light/dark) at Cold Spring Harbor Laboratory. Day and night temperatures were set to 25°C and 18°C, respectively, with a relative humidity of 40-60%. Shoot apical meristems were imaged with a Nikon SMZ1500 microscope for all species and stages as previously described (Park et al. 2012).

Tissue collection and RNA sequencing

Meristem tissue was collected and RNA extracted using previously published protocols (Park et al. 2012). In brief, seedlings (about 3 cm in length) from all species were fixed in 100% acetone, vacuum infiltrated, and meristems carrying up to P2 were collected by microdissection under a stereomicroscope. Each biological replicate consisted of 50 or more pooled meristems from individual plants. Meristems were dried for 3 minutes at room temperature to remove residual acetone and ground in a mixer mill MM300 (Retsch). Total RNA was extracted with PicoPure™ RNA Extraction kits (Arcturus, KIT0204) and DNase (Qiagen) treated. We assessed RNA quality by gel electrophoresis or Bioanalyzer 2100 (Agilent), only retaining high quality RNA for library preparation and semi-quantitative RT-PCR. To validate stage and meristem specificity of samples, we assayed several previously published stage specific marker genes via semi-quantitative RT-PCR. Marker genes included orthologs of the TM and SYM specific *SELF PRUNING* (*SP*), *SINGLE FLOWER TRUSS* (*SFT*) orthologs expressed in the VM to the TM, *APETALA1* and *SEPALLATA2* (*AP1* and *SEP2*) orthologs as TM, SIM, and FM markers, and *S*

and *AN* orthologs for TM/SIM and FM specific markers respectively (Park et al. 2012). Loading controls consisted of *UBIQUITIN* (*S. lycopersicum*, *S. peruvianum*, and *S. prinophyllum*), *C. annuum Ubiquitin Extension Protein* (*CaUEP*), and *N. benthamiana Actin* (*NbACT*). Primer sequences are available in Supplemental Table S12.

Two biological replicates per meristem stage and species were used for transcriptome profiling. Poly-A containing mRNA (20~80 ng), purified from 1-3 ug total RNA with Invitrogen Dynabeads, was used for mRNA-seq library construction according to the ScriptSeq v2 RNA library preparation kit (Epicentre) or NEBNext® Ultra™ RNA Library Prep Kit (New England Biolabs) with barcodes for transcriptome profiling (Supplemental Table S3). Library amplification was performed through minimal PCR amplification (10-15 cycles) with Illumina compatible PE PCR primers. Final library quality and size distribution was determined by Bioanalyzer 2100 (Agilent) and quantified with the KAPA Library Quantification Kit (Kapa Biosystem). For *S. prinophyllum* and *C. annuum*, we pooled four barcoded libraries per lane for sequencing on the Illumina HiSeq 2000 platform (100 bp paired end runs). Transcriptome sequence for *N. benthamiana* was obtained using one lane per sample on the Illumina GAIIx platform. The reads for *S. peruvianum* and *S. lycopersicum* came from previous work again using one sample per lane of Illumina GAIIx (Park et al. 2012). All GAIIx sequencing consisted of 50 bp paired end runs. To minimize the impact of using two Illumina platforms, we only assessed dynamic expression within species (platform), and absolute expression levels (counts or CPM) were never directly compared between species for matched stages. Rather, comparisons between species were made with standardized expression dynamics. Importantly, our results from the transcriptome analysis were supported by our semi-qRT-PCR and *in situ* analyses.

Transcriptome assembly and quantification

All reads were assessed for overall quality using the FastQC tool v0.11.2 (Andrews 2014) and trimmed based on quality using Trimmomatic v0.32 (Bolger et al. 2014) (HiSeq 2000 read

parameters: ILLUMINACLIP:TruSeq3-PE-2.fa:2:40:15:1:FALSE LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36 ; GAIIx read parameters: ILLUMINACLIP:TruSeq2-PE.fa:2:30:10:1:FALSE LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36 TOPHRED33). Remaining paired reads for wild tomato, domesticated tomato, and tobacco were then aligned to their respective reference genome sequences (Tomato Genome Consortium 2012; Bombarely et al. 2012) using the splice aware Tophat2 v2.0.12 (Kim et al. 2013) aligner (parameters: --b2-very-sensitive --read-mismatches 2 --read-edit-dist 2 --min-anchor 8 --splice-mismatches 0 --min-intron-length 50 --max-intron-length 50000 --max-multihits 20). Notably, *S. peruvianum* was aligned to the reference tomato genome SL2.50 with additional mismatches allowed (--read-mismatches 4 –read-edit-dist 4) to accommodate the cross species alignments. Unique paired reads aligned to annotated gene features were then counted with HTSeq-count v0.6.0 (parameters: --mode=union --stranded=no) (Anders et al. 2015).

Two species either completely or partially lacked reference genome sequences and were quantified by alignment to transcriptome sequences instead. The non-model *S. prinophyllum* completely lacked reference sequences and thus a Trinity *de novo* transcriptome assembly (r20140717; parameters: --JM 400G --trimmomatic --normalize_reads) was performed using all reads as input (Grabherr et al. 2011). Assembled transcripts were then scanned for open reading frames (Trinity TransDecoder default) and resulting CDS sequences used as reference transcripts for alignment. Only CDS sequences that were included in orthologous gene groups were used in the alignment. Pepper lacked a complete reference genome, so alignment was done to the reference transcriptome v1.55 (Kim et al. 2014). Trimmed reads were aligned to transcriptomes with Bowtie2 v2.2.3 (Langmead and Salzberg 2012; Langmead et al. 2009) (parameters: -N0 -L22 --minins 10 --maxins 500 --fr --score-min L,-0.6,-0.6) and concordant read pair alignments counted with a bash script.

Orthologous gene groups (orthogroups) were defined using the Orthologous MAtrix (OMA Version 0.99z.3) pipeline (Dessimoz et al. 2005; Roth et al. 2008) with reference Heinz

tomato (ITAG2.4) proteins (Tomato Genome Consortium 2012), reference pepper (v1.55) proteins (Kim et al. 2014), reference tobacco (v0.4.4) proteins (Bombarely et al. 2012), and protein sequences extracted from *de novo* assembled transcripts of the *S. prinophyllum* species using the Trinity pipeline (Grabherr et al. 2011). *S. peruvianum* proteins were not included as a separate group of proteins as divergence between tomato and *S. peruvianum* is minimal. Domesticated tomato (*S. lycopersicum*) genes were used as a foundation for building orthogroups by considering all pairwise hits with domesticated tomato from other species. A cumulative orthogroup expression value in CPM was calculated *N. benthamiana*, *C. annuum*, and *S. prinophyllum* by averaging all potential hits excluding geneids making up less than 10% of maximum observed expression. Specific orthogroup content in terms of geneids can be found in Supplemental Table S8.

Statistical analyses for heterochrony and modified maturation schedules

All statistical analyses of gene expression were conducted in R (R Development Core Team 2013) expect for GO term enrichment analyses that used the agriGO toolkit (Du et al. 2010). For cross species comparisons, orthogroup expression was examined after z-score normalization (normalized expression) or scaling from zero to one (scaled expression) within species. Hierarchical clustering, heatmaps, PCA, transcriptome expression divergence, and *k*-means clustering were computed with normalized expression values. Gene-by-gene comparisons used scaled values to examine precocious expression.

Significant differential expression for all potential pairwise meristem stage comparisons was determined separately for each species by edgeR (Robinson et al. 2010) using 2-foldchange, average 1 CPM, and FDR $\leq$ 0.10 cutoffs. Consistent dynamic expression of an orthogroup was defined in the main text as differential expression in four of the five species. For orthogroups with multiple genes in a given species, differential expression was only required for one geneid. For the purpose of clustering, heatmaps, PCA, and transcriptome distance, biological replicates were

averaged. Heatmaps were produced from merged normalized expression matrices in R with the gplots package heatmap.2 function (Warnes et al. 2015). PCA analyses for PSM stages (Fig. 2B and D) as well as SIM stages (Fig. 4) also used normalized expression matrices with the prcomp function in R. Pairwise transcriptome distance for each developmental stage between species was calculated as Euclidean distance from Pearson correlation of normalized expression and a one-sided Kolmogorov-Smirnov test was used to compare the combined transitional stages (LVM and TM) distances versus MVM and FM. Heatmaps, PCA, and transcriptome distance produced for additional definitions of dynamic expression including dynamic expression in one species, two species, three species, all species, and domesticated tomato are consistent with results shown in the main figure (Supplemental Fig. S7-S9).

Analyses exploring heterochronic shifts in comparison to tomato were conducted using both normalized and scaled expression. Initial comparisons used *k*-means clustering of normalized orthogroup expression in a two-step process. First, twelve clusters were calculated from normalized tomato expression. Then, 10 sub-clusters for non-tomato orthogroup expression were calculated within each original tomato cluster. This produced a total of 120 cluster:sub-cluster groups, 12 tomato clusters * 10 sub-clusters, for each species (Supplemental Fig. S10). Precocious expression in tobacco and pepper was examined with gene-by-gene comparisons of scaled expression. Only orthogroups where tomato expression was 2-fold higher than any other developmental stage were used to focus the analysis on stage specific genes. We additionally used several CPM cutoffs (1, 2, 3, 4, and 5) for the non-tomato orthogroup and several foldchange cutoffs (1, 1.5, and 2) for precocious expression.

in situ hybridization

We used non-radioactive mRNA *in situ* hybridization to assess localization and intensity of expression for *S* and *AN* orthologs (Jackson 1992). According to stage definitions described above, shoot apices were dissected and fixed in 4% paraformaldehyde with 0.3% Triton X-100.

Late transition meristems (LTM), intermediate to TM and FM stages, were collected for a more granular dissection of *S* and *AN* expression patterns. *In vitro* transcribed RNA probes for *AN* orthologs (*SlAN, SperuAN, CaAN,* and *NbAN*) were generated from full-length cDNA. The probes for *S* orthologs were synthesized from 5' CDS fragments (~600bp length). Transcripts were detected using the DIG *in situ* hybridization system (Roche). Primer sequences used for cloning templates are provided in Supplemental Table S12.

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