

Supporting Information

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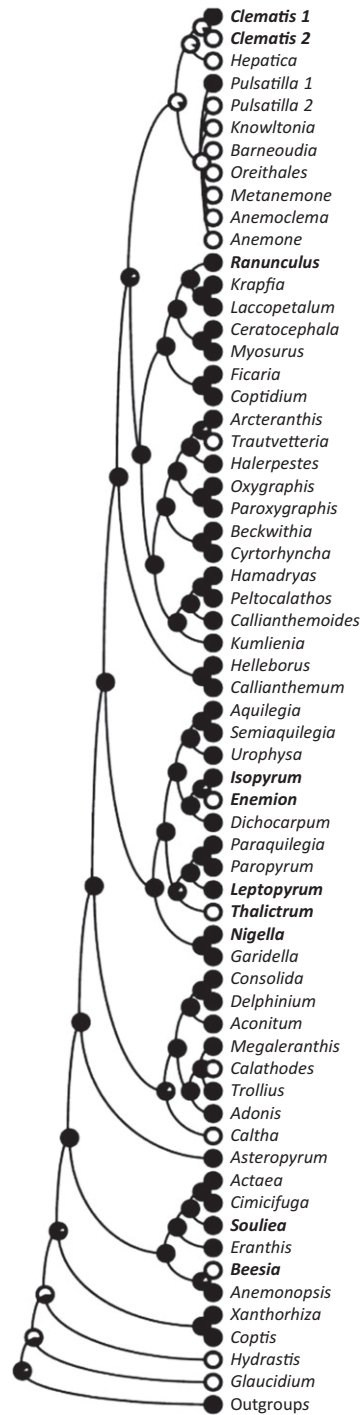


Fig. S1. Parallel petal losses within the Ranunculaceae. Filled and open circles indicate the character states of being petalous and apetalous, respectively. The probabilities of being petalous, which were inferred using the maximum likelihood method in Mesquite version 2.72, are indicated for each interior node. Note that in at least seven different lineages (excluding the two basal-most genera, *Glaucidium* and *Hydrastis*), apetalous genera all have petalous ancestors.

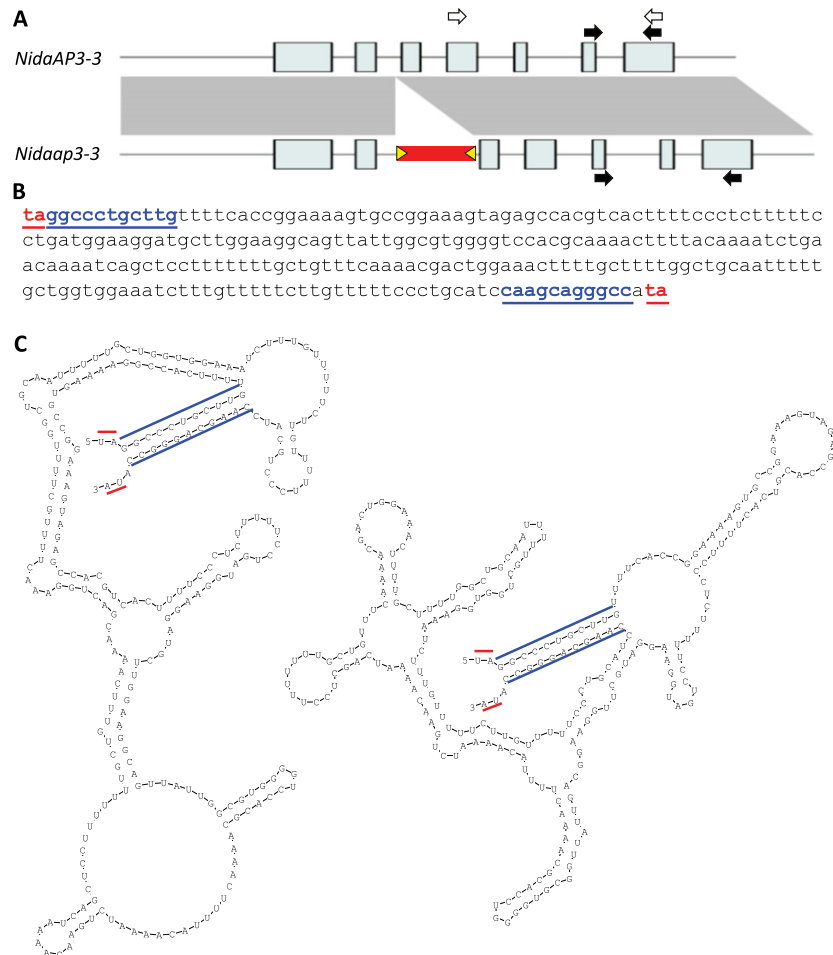


Fig. S2. Comparison of the functional and nonfunctional *AP3-3* alleles in *Nigella*. (A) Exon–intron structures of *NidaAP3-3* (Upper) and *Nidaap3-3* (Lower). Open and closed arrows indicate the positions from which primers used for *in situ* hybridization and qRT-PCR analyses, respectively, were derived (details given in Tables S1 and S3). The red block with two yellow triangles at both sides indicates the predicated *Gypsy/Ty3*-like miniature inverted-repeat transposable element (MITE) in *Nidaap3-3*. (B) Sequence of the predicted MITE, with the reverse complementary sequences and the “TA” repeats highlighted by blue and red bars, respectively. (C) Secondary structures of the MITE, predicted in DNAMAN 7.0 according to its minimum free energy.

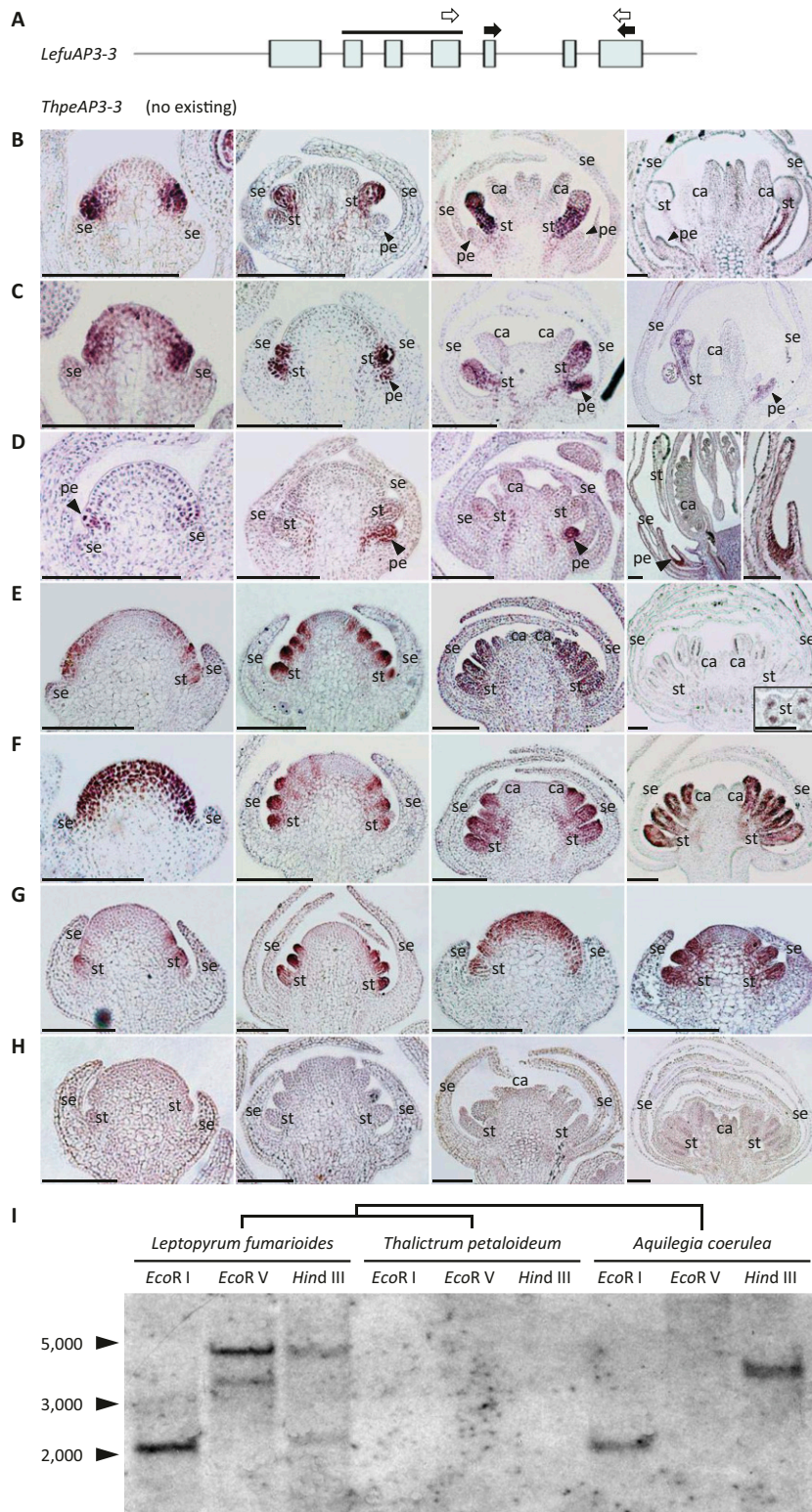


Fig. S3. The *AP3-3* genes from *Leptopyrum* and *Thalictrum*. (A) Exon–intron structure of *LefuAP3-3*. Open and closed arrows indicate the positions from which primers used for in situ hybridization and qRT-PCR analyses, respectively, were derived (Tables S1 and S3 give details). The black line indicates the region used as probe in Southern hybridization. (B–H) In situ hybridization of *LefuAP3-1* (B), *LefuAP3-2* (C), and *LefuAP3-3* (D) in *Leptopyrum*, *ThpeAP3-1* (E) and *ThpeAP3-2b* (F) in *Thalictrum*, and *LefuAP3-1* (G, left two pictures), *LefuAP3-2* (G, right two pictures), and *LefuAP3-3* (H) in *Thalictrum*. Arrowheads indicate petals; se, st, and ca refer to sepals, stamens, and carpels, respectively. (Scale bar, 50 μ m.) (I) Southern hybridization of the *LefuAP3-3* probe in three species. For Southern hybridization, genomic DNA was extracted from fresh leaves of each species and digested with restriction enzymes *EcoR* I, *EcoR* V, and *Hind* III. A 458-bp genomic DNA fragment covering the second to fourth exons of *Leptopyrum AP3-3* was labeled with a thermostable alkaline phosphatase enzyme (GE Healthcare) and used as a probe. The whole process was performed according to the instruction of Amersham Gene Images Alkphos Direct Labeling and Detection System (GE Healthcare).

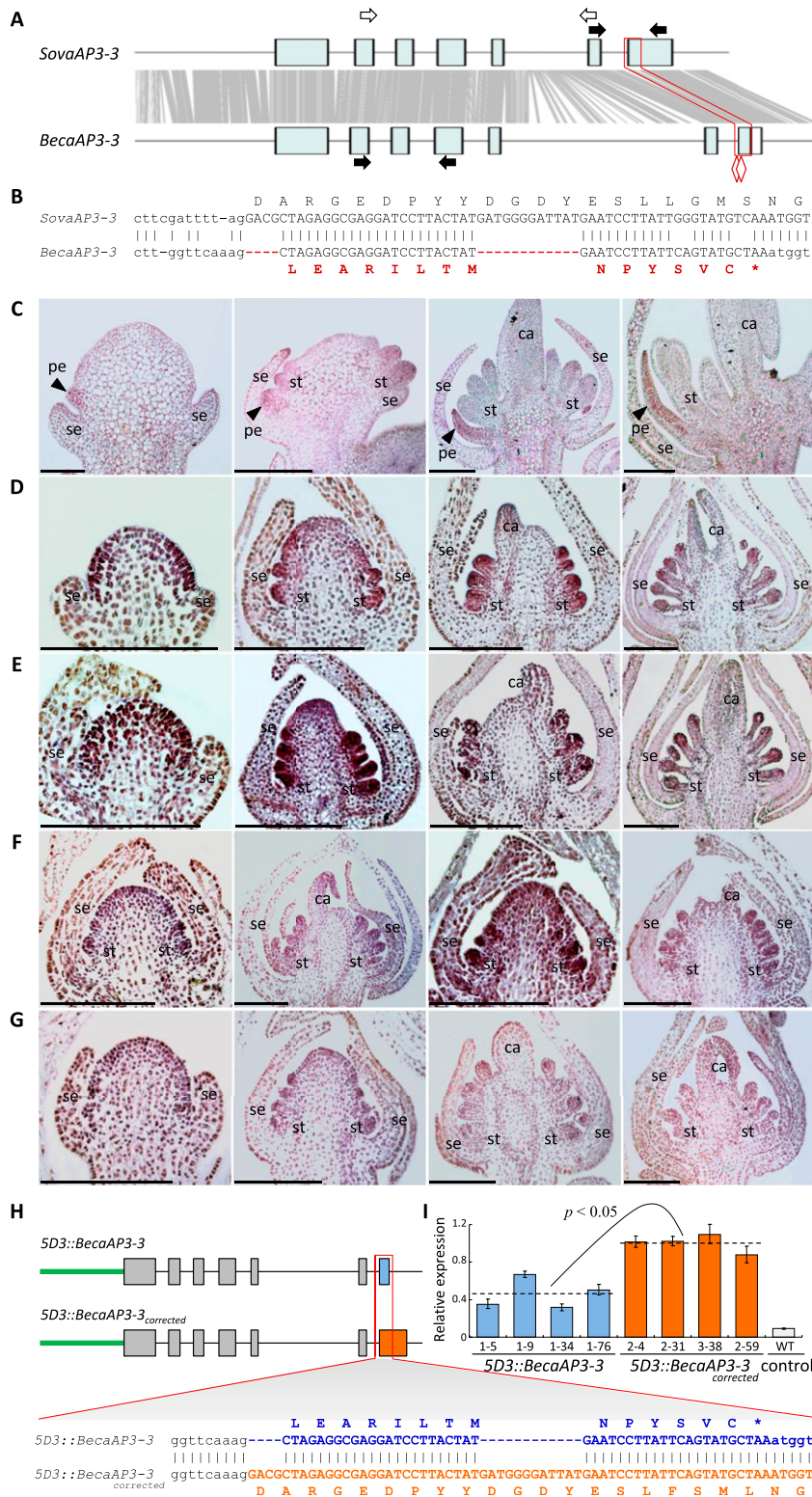


Fig. S4. The *AP3-3* genes from *Souliea* and *Beesia*. (A) Exon-intron structures of *SovaAP3-3* and *BecaAP3-3*. Open and closed arrows indicate the positions from which primers used for in situ hybridization and qRT-PCR analyses, respectively, were derived (Tables S1 and S3 give details). (B) Alignment of the regions included in the red polygon. Uppercase letters denote exonic sequences; lowercase letters denote intronic or 3' UTR sequences. Corresponding amino acids are shown above and below the exons. (C–G) In situ hybridization of *SovaAP3-3* (C) in *Souliea*, *BecaAP3-1* (D), and *BecaAP3-2* (E) in *Beesia* and *SovaAP3-1* (F, left two pictures), *SovaAP3-2* (F, right two pictures), and *SovaAP3-3* (G) in *Beesia*. Arrowheads indicate petals; se, st, and ca refer to sepals, stamens, and carpels, respectively. (Scale bars, 50 μ m.) (H) Schematics of the constructs *5D3::BecaAP3-3* and *5D3::BecaAP3-3_{corrected}*, with their actual sequences shown underneath. The green line denotes the promoter of the *Arabidopsis AP3* gene, *5D3*, and the blue and orange boxes indicate the differentiated exons between the two genes, respectively. (I) Expression levels of the two constructs in different transgenic lines of *Arabidopsis* as revealed by qRT-PCR analyses. Dashed lines show the average expression level of the two constructs. The expression of *BecaAP3-3* in the untreated *Arabidopsis* plants was used as a negative control.

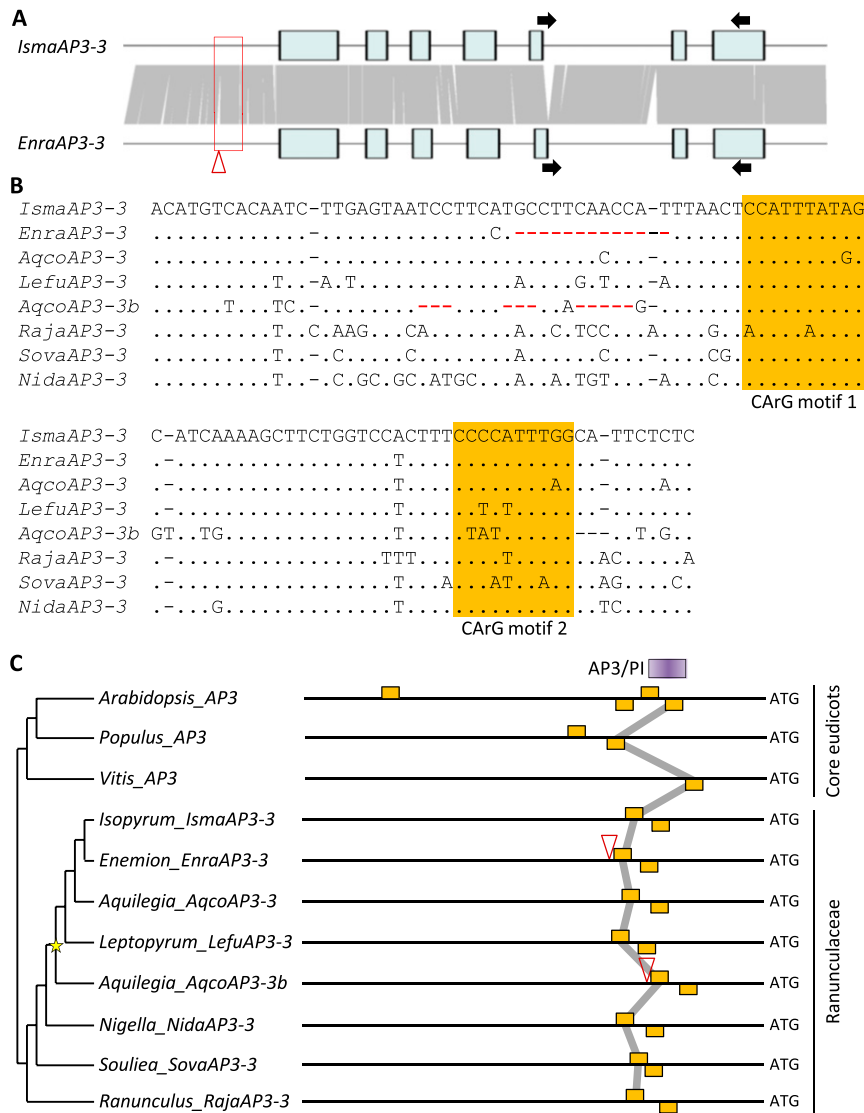


Fig. S5. The *AP3-3* genes from *Isopyrum* and *Enemion*. (A) Exon-intron structures of *IsmaAP3-3* and *EnraAP3-3*. Arrows indicate the positions from which primers used for qRT-PCR analyses were derived (Tables S1 and S3 give details). (B) Alignment of the promoter region of *AP3* orthologs covered with the red rectangle in A. The two yellow boxes show the highly conserved CARg-box motifs, named CARg motif1 and CARg motif2, respectively. (C) Conservativeness of the CARg motif1 across eudicots. The homolog of the CARg motif1 in *Arabidopsis*, CARg3, has been demonstrated to be the binding sites of the AP3/PI heterodimer and is indicated by a purple rectangle. The yellow star on the phylogenetic tree stands for inferred gene duplication event.

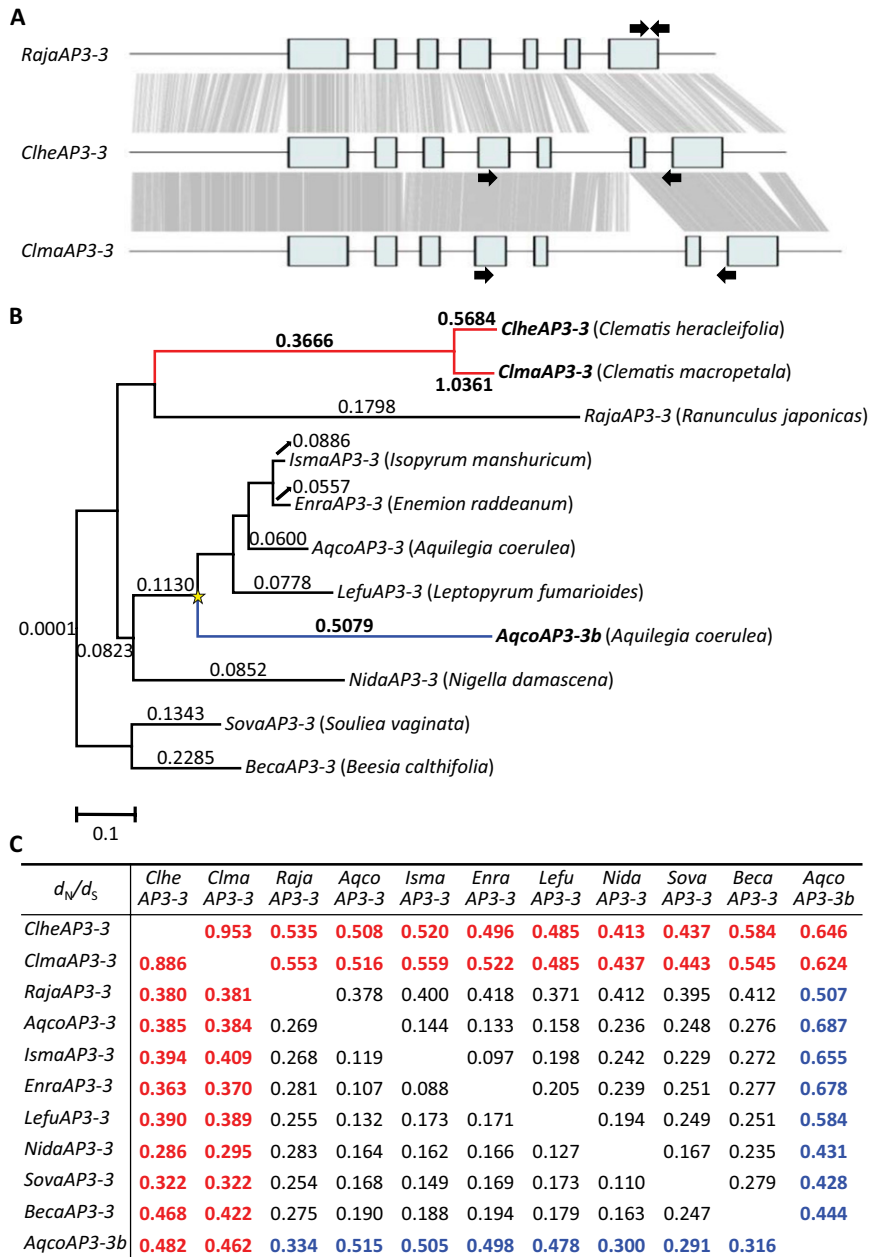


Fig. 56. The AP3-3 genes in *Ranunculus* and *Clematis*. (A) Exon-intron structures of *RajaAP3-3*, *ClheAP3-3*, and *ClmaAP3-3*. Arrows indicate the positions from which primers used for qRT-PCR analyses were derived (Tables S1 and S3 give details). (B) Phylogenetic relationships among AP3-3-like genes, with ω values, which represent the ratios of nonsynonymous substitution rate (d_N) versus synonymous substitution rate (d_S) and were estimated in PAML using the branch model, being indicated for each branch. Branches leading to the *Clematis* AP3-3 genes are in red, whereas that to *AqcoAP3-3b*, which is no longer functional, is in blue. The yellow star stands for the inferred gene duplication event. (C) Pairwise comparisons of AP3-3-like genes in terms of their ω values, which were calculated in MEGA 5 with the Kumar (Kimura 2-para; Lower) and modified Nei-Gojobori (proportion; Upper) models.

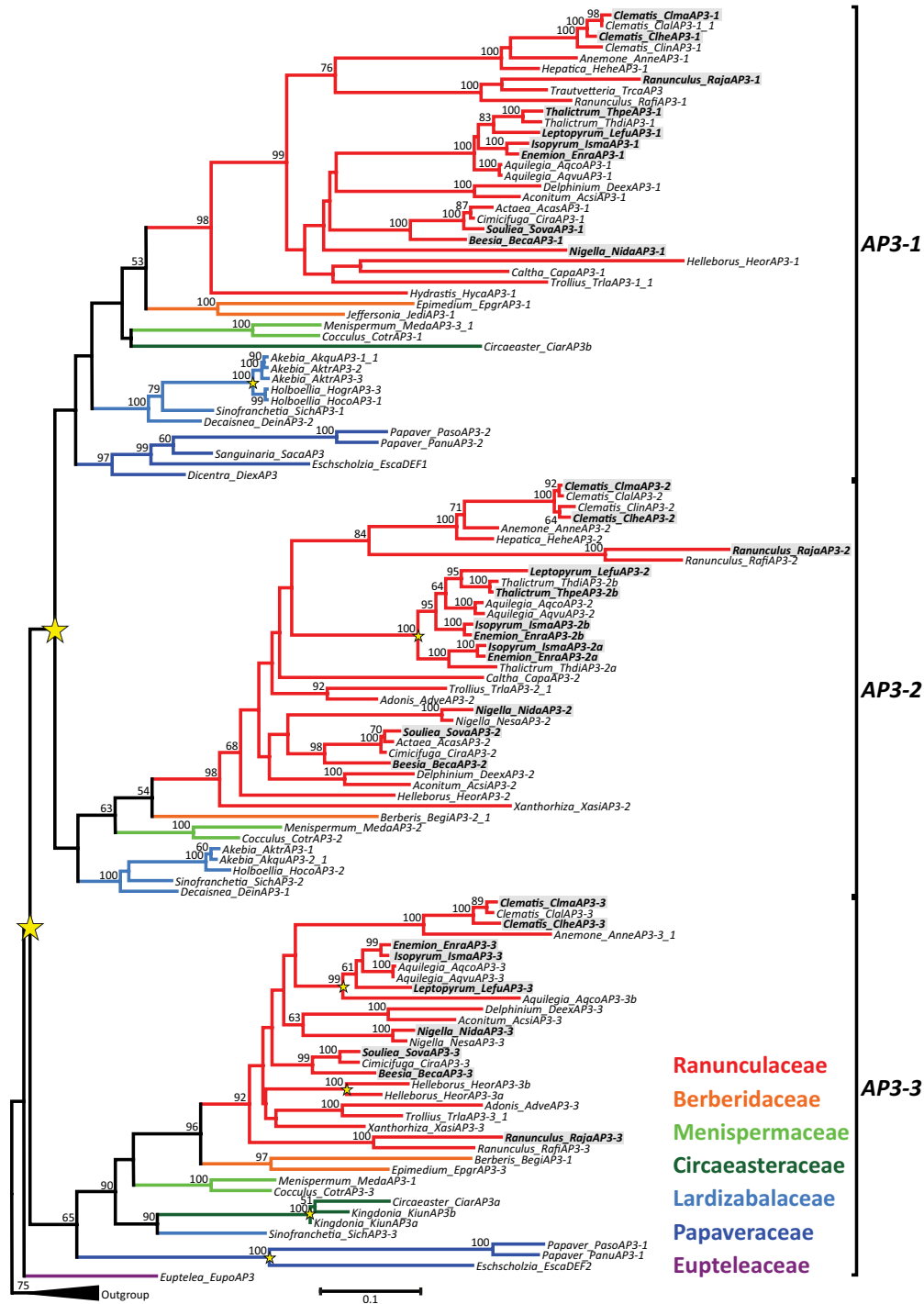


Fig. S7. Phylogenetic relationships of AP3-like genes from the Ranunculales. The tree was reconstructed in PhyML by using the maximum likelihood method, with higher-than-50% bootstrap supports indicated for each node. Yellow stars stand for the inferred gene duplication events. Sequences isolated in this study are highlighted in bold with gray shadow. Information on sequences is given in Table S4.

Table S1. Primers used in this study

Taxon/species and purpose	Gene name	Primer name
Ranunculaceae		
cDNA synthesis		polyT
cDNA amplification*	<i>AP3</i> -like	B1 <i>R</i> _AP3-1 <i>R</i> _AP3-2 <i>R</i> _AP3-3 Adaptor <i>R</i> _ACTIN
<i>Nigella damascena</i>		
qRT-PCR	<i>ACTIN</i>	<i>R</i> _ACTIN
qRT-PCR	<i>NidaAP3-1</i> <i>NidaAP3-2</i> <i>NidaAP3-3</i>	<i>NidaAP3-1</i> _RT <i>NidaAP3-2</i> _RT <i>NidaAP3-3</i> _RT
In situ hybridization	<i>NidaAP3-3</i>	<i>NidaAP3-3</i> _In
<i>Leptopyrum fumaroides</i>		
qRT-PCR	<i>LefuAP3-1</i> <i>LefuAP3-2</i> <i>LefuAP3-3</i>	<i>LefuAP3-1</i> _RT <i>LefuAP3-2</i> _RT <i>LefuAP3-3</i> _RT
In situ hybridization	<i>LefuAP3-1</i> <i>LefuAP3-2</i> <i>LefuAP3-3</i>	<i>LefuAP3-1</i> _In <i>LefuAP3-2</i> _In <i>LefuAP3-3</i> _In
Southern hybridization	<i>LefuAP3-3</i>	<i>LefuAP3-3</i> _S
<i>Thalictrum petaloideum</i>		
qRT-PCR	<i>ThpeAP3-1</i> <i>ThpeAP3-2b</i>	<i>ThpeAP3-1</i> _RT <i>ThpeAP3-2b</i> _RT
In situ hybridization	<i>ThpeAP3-1</i> <i>ThpeAP3-2b</i>	<i>ThpeAP3-1</i> _In <i>ThpeAP3-2b</i> _In
<i>Souliea vaginata</i>		
qRT-PCR	<i>SovaAP3-1</i> <i>SovaAP3-2</i> <i>SovaAP3-3</i>	<i>SovaAP3-1</i> _RT <i>SovaAP3-2</i> _RT <i>SovaAP3-3</i> _RT
In situ hybridization	<i>SovaAP3-1</i> <i>SovaAP3-2</i> <i>SovaAP3-3</i>	<i>SovaAP3-1</i> _In <i>SovaAP3-2</i> _In <i>SovaAP3-3</i> _In
<i>Beesia calthifolia</i>		
In situ hybridization	<i>BecaAP3-1</i> <i>BecaAP3-2</i>	<i>BecaAP3-1</i> _In <i>BecaAP3-2</i> _In
qRT-PCR	<i>BecaAP3-1</i> <i>BecaAP3-2</i> <i>BecaAP3-3</i>	<i>BecaAP3-1</i> _RT <i>BecaAP3-2</i> _RT <i>BecaAP3-3</i> _RT
<i>Isopyrum manshuricum</i>		
qRT-PCR	<i>IsmaAP3-1</i> <i>IsmaAP3-2b</i> <i>IsmaAP3-3</i>	<i>IsEnAP3-1</i> _RT <i>IsEnAP3-2</i> _RT <i>IsEnAP3-3</i> _RT
<i>Enemion raddeanum</i>		
qRT-PCR	<i>EnraAP3-1</i> <i>EnraAP3-2b</i> <i>EnraAP3-3</i>	<i>IsEnAP3-1</i> _RT <i>IsEnAP3-2</i> _RT <i>IsEnAP3-3</i> _RT
<i>Ranunculus japonicus</i>		
qRT-PCR	<i>RajaAP3-1</i> <i>RajaAP3-2</i> <i>RajaAP3-3</i>	<i>RajaAP3-1</i> _RT <i>RajaAP3-2</i> _RT <i>RajaAP3-3</i> _RT
<i>Clematis heracleifolia</i>		
qRT-PCR	<i>ClheAP3-1</i> <i>ClheAP3-2</i> <i>ClheAP3-3</i>	<i>ClAP3-1</i> _RT <i>ClAP3-2</i> _RT <i>ClAP3-3</i> _RT
<i>C. macropetala</i>		
qRT-PCR	<i>ClmaAP3-1</i> <i>ClmaAP3-2</i> <i>ClmaAP3-3</i>	<i>ClAP3-1</i> _RT <i>ClAP3-2</i> _RT <i>ClAP3-3</i> _RT
<i>Arabidopsis</i>		
Genotyping	<i>BecaAP3-3</i> <i>BecaAP3-3</i> <i>BecaAP3-3</i>	Bc33_GT Bc33 _{corrected} _GT 5D3::Bc33_GT
qRT-PCR	<i>ACTIN</i>	<i>At</i> _ACTIN_RT

*For amplification of *AP3*-like cDNA, B1 was used for the first round of PCR and *R*_AP3-1, *R*_AP3-2, and *R*_AP3-3 were used for the second round of PCR.

Table S2. Information on the species sampled in this study

Species	Location	Voucher
<i>Besia calthifolia</i>	33°38' N, 106°52' E, Hanzhong, Shaanxi, China	Chunce Guo 0804-103
<i>Clematis heracleifolia</i>	39°59' N, 116°12' E, Institute of Botany, Chinese Academy of Sciences (CAS), Beijing, China	Chunce Guo 0807-16
<i>Clematis macropetala</i>	40°02' N, 115°29' E, Dongling Mountain, Beijing, China	Chunce Guo 0806-75
<i>Enemion raddeanum</i>	40°42' N, 126°05' E, Tonghua, Jilin, China	Rui Zhang 1004-33
<i>Isopyrum manshuricum</i>	40°43' N, 126°01' E, Tonghua, Jilin, China	Rui Zhang 1004-27
<i>Leptopyrum fumarioides</i>	45°43' N, 126°37' E, Harbin, Heilongjiang, China	Chunce Guo 0805-56
<i>Nigella damascena</i>	Cultivated in Institute of Botany, CAS, Beijing, China	Wengen Zhang 1012-06
<i>N. damascena</i> "Double Sepal"	Cultivated in Institute of Botany, CAS, Beijing, China	Wengen Zhang 1012-07
<i>Ranunculus japonicus</i>	39°59' N, 116°12' E, Institute of Botany, CAS, Beijing, China	Rui Zhang 1205-07
<i>Souliea vaginata</i>	33°58' N, 107°45' E, Taibai Mountain, Shaanxi, China	Chunce Guo 0804-114
<i>Thalictrum petaloideum</i>	40°02' N, 115°29' E, Dongling Mountain, Beijing, China	Rui Zhang 1006-05

Table S3. Sequence information on primers listed in Table S2

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
5D3::Bc33_GT	CGTTAAGTTTGTCCACCGTC	GATCAATCAACCTCGTAGTG
Adaptor		CCGGATCCTCTACAGCGGCCGC
<i>At_ACTIN_RT</i>	TCGTTTGTGGGAATGGAAGC	GATACCTGAGAACATAGTGGT
B1	AACAGGCAGGTSACCTAYTC	
Bc33_GT	ATACATACCAGCGTCTCACC	CAGAACCGTCCAGATCTTG
Bc33 _{corrected} _GT	GAGAAAGTGTCCAGTTGC	GGATTCATAATCCCCATC
<i>BecaAP3-1_In</i>	GGAAAGTTTGGTGTGTTAGG	TCACGAAGATTGGGTGG
<i>BecaAP3-1_RT</i>	AGGAAGTTTGGTGTGTTAGG	AGATTGGGTGGCTTGGTTG
<i>BecaAP3-2_In</i>	CACAGACCGATACATACAAG	ACCATACGAGGCATAAAC
<i>BecaAP3-2_RT</i>	CATTTGAAGGGAGAGACGAG	AAACCATACGAGGCATAAAC
<i>BecaAP3-3_RT</i>	ATACATACCAGCGTCTCACC	CAGAACCGTCCAGATCTTG
<i>CIAP3-1_RT</i>	CACTAGCCAACCATCAAGG	TCCATTTCCACTCAAGCCAG
<i>CIAP3-2_RT</i>	AAGAGGGAGATACGTCGTG	CCATAGGAGCAGTAGAGAG
<i>CIAP3-3_RT</i>	TGGGAAGGAAAGTCTTGATG	TCCGTAGTAAGCAACCTCAC
<i>IsEnAP3-1_RT</i>	ATTGTGTGGTCTTGAGCAAC	TCCTTCATGATCGGCTAGTG
<i>IsEnAP3-2_RT</i>	CCATAACAGTCTGATACGTG	CATCCTGAAGATCGGCTGAC
<i>IsEnAP3-3_RT</i>	CCTACAGGAAGAAACTAAGG	TGAATGTTGTGTTGGCTTGG
<i>LefuAP3-1_In</i>	AAGGGTTGGTGGTGTATGA	AAGATTAGGCTGGCTTGG
<i>LefuAP3-1_RT</i>	ATCATGAAGGATTGTCTGCAC	CAACCTAGTCGGAGATTCTC
<i>LefuAP3-2_In</i>	CTGAAGGAGACTAACAACAAAC	CAAGTGATGGAAGCGGA
<i>LefuAP3-2_RT</i>	ATGCAGACTTCCGCTTTCC	ATCCATATGGTCTTCTCATCCT
<i>LefuAP3-3_In</i>	CAAGATTGGATGAGTCAGT	CGATAAGAGACAAGGTGTG
<i>LefuAP3-3_RT</i>	CATGATTGCTACACAGACTG	CAAACGATAAGAGACAAGGTG
<i>LefuAP3-3_S</i>	CAGAACAAAGAAAGTATATGATC	ATAACCTTTCGATCGCGAACCCAG
<i>NidaAP3-1_RT</i>	CTCATTGAGGATTGTTAGCC	GGTGGTGTGGGTGGTTG
<i>NidaAP3-2_RT</i>	AGTGTGTTGCTTTCAGGTTGC	CATAACTACTTAGTCCAGAGAG
<i>NidaAP3-3_In</i>	GATTGGAGAAAGTGTGATGAC	TGAAGATTAGGTTGGCTTGG
<i>NidaAP3-3_RT</i>	GAACTTGGCAGAAACACATAC	AGATTAGGTTGGCTTGGTTG
polyT		CCGGATCCTCTAGAGCGGCCGC (T) ¹⁷
<i>R_ACTIN</i>	CCGTTCTGCTGTGGTTGTG	GTGTTGGACTCTGGTGTATGG
<i>R_AP3-1</i>	GTTCTTTGTGATGCGYAGGTT	
<i>R_AP3-2</i>	CTYACTGTTCTYTYGYATGCT	
<i>R_AP3-3</i>	CTYACTGTTCTTTGTGATGC	
<i>RajaAP3-1_RT</i>	GTGGACTATCTGCACTTGAAC	ACCTCCCATTCTACTGATTG
<i>RajaAP3-2_RT</i>	CCCAGTATTCATTTGTTTCAGC	TAAGAGCCATAGTCTTGAGC
<i>RajaAP3-3_RT</i>	GTTTTCTATGGTCTTCAGCC	CATCACCTGTTAACGCCAATG
<i>SovaAP3-1_In</i>	TTGCTGAGTTGCGTGGTC	CGAAGATTGGGTGGCTG
<i>SovaAP3-1_RT</i>	GGGATCTCTACAATGAACTG	ATCTACTCAACCAAGTCGG
<i>SovaAP3-2_In</i>	CGTGATTGGTACTTCGACAG	CTCTATTCATTCAGCAAGACG
<i>SovaAP3-2_RT</i>	ACTACGCACTCATTGATCAG	AAGCAAGACGTGAACCATAAC
<i>SovaAP3-3_In</i>	CCAACAGGTCAATGGAGT	ATCAGATGTGTGTGGCTG
<i>SovaAP3-3_RT</i>	GCAAGAGACGCACACACATC	ATCGTAGGATCCATAGGTTTC
<i>ThpeAP3-1_In</i>	TTGAAGAATTGTGCGGCTTTGAGC	TGGAATCCATATCCTCCATCACCA
<i>ThpeAP3-1_RT</i>	TGAGGGAGAATATGCACTAG	GATTAGGCTGGCTTGGTTGC
<i>ThpeAP3-2b_In</i>	GTTGAGTGTGTTGTAATC	GTCTAGATGTGGAACGATAAC
<i>ThpeAP3-2b_RT</i>	ACCAAAGCTGCAGTCCAATC	GGAACGATAACATGATATCCAG

Table S4. Information on the AP3-like genes used in this study

Taxon and species*	Gene name*	Accession no.†	Source
Ranunculaceae			
<i>Aconitum sinomontanum</i>	<i>AcsiAP3-1</i>	EU481818	1
	<i>AcsiAP3-2</i>	EU481817	1
	<i>AcsiAP3-3</i>	EU481816	1
<i>Actaea asiatica</i>	<i>AcasAP3-1</i>	HQ647375	2
	<i>AcasAP3-2</i>	HQ647376	2
<i>Adonis vernalis</i>	<i>AdveAP3-2</i>	HQ694800	2
	<i>AdveAP3-3</i>	HQ694799	2
<i>Anemone nemorosa</i>	<i>AnneAP3-1</i>	AY162841	3
	<i>AnneAP3-2</i>	AY162842	3
	<i>AnneAP3-3_1</i>	AY162843	3
<i>Aquilegia coerulea</i>	<i>AqcoAP3-1</i>	Aquca_006_00074 [‡]	—
	<i>AqcoAP3-2</i>	Aquca_006_00072 [‡]	—
	<i>AqcoAP3-3</i>	Aquca_007_00336 [‡]	—
	<i>AqcoAP3-3b</i>	HQ694798	2
<i>Aquilegia vulgaris</i>	<i>AqvuaAP3-1</i>	EF489478	4
	<i>AqvuaAP3-2</i>	EF489477	4
	<i>AqvuaAP3-3</i>	EF489476	4
<i>Beesia calthifolia</i>	<i>BecaAP3-1</i>		This study
	<i>BecaAP3-2</i>		This study
	<i>BecaAP3-3</i>		This study
<i>Caltha palustris</i>	<i>CapaAP3-1</i>	EU481813	1
	<i>CapaAP3-2</i>	EU481812	1
<i>Cimicifuga racemosa</i>	<i>CiraAP3-1</i>	AY162862	3
	<i>CiraAP3-2</i>	AY162863	3
	<i>CiraAP3-3</i>	AY162864	3
<i>Clematis alpina</i>	<i>ClalAP3-1_1</i>	EU481808	1
	<i>ClalAP3-2</i>	EU481807	1
	<i>ClalAP3-3</i>	EU481806	1
<i>Clematis integrifolia</i>	<i>ClinAP3-1</i>	AY162870	3
	<i>ClinAP3-2</i>	AY162871	3
<i>Clematis heracleifolia</i>	<i>ClheAP3-1</i>		This study
	<i>ClheAP3-2</i>		This study
	<i>ClheAP3-3</i>		This study
<i>Clematis macropetala</i>	<i>ClmaAP3-1</i>		This study
	<i>ClmaAP3-2</i>		This study
	<i>ClmaAP3-3</i>		This study
<i>Delphinium exaltatum</i>	<i>DeexAP3-1</i>	EU481804	1
	<i>DeexAP3-2</i>	EU481803	1
	<i>DeexAP3-3</i>	EU481802	1
<i>Enemion raddeanum</i>	<i>EnraAP3-1</i>		This study
	<i>EnraAP3-2a</i>		This study
	<i>EnraAP3-2b</i>		This study
	<i>EnraAP3-3</i>		This study
<i>Helleborus orientalis</i>	<i>HeorAP3-1</i>	AY162874	3
	<i>HeorAP3-2</i>	AY162875	3
	<i>HeorAP3-3a</i>	AY162876	3
	<i>HeorAP3-3b</i>	AY162877	3
<i>Hepatica henryi</i>	<i>HeheAP3-1</i>	HQ647378	2
	<i>HeheAP3-2</i>	HQ647379	2
<i>Hydrastis canadensis</i>	<i>HycaAP3-1</i>	EU481800	1
<i>Isopyrum manshuricum</i>	<i>IsmaAP3-1</i>		This study
	<i>IsmaAP3-2a</i>		This study
	<i>IsmaAP3-2b</i>		This study
	<i>IsmaAP3-3</i>		This study
<i>Leptopyrum fumarioides</i>	<i>LefuAP3-1</i>		This study
	<i>LefuAP3-2</i>		This study
	<i>LefuAP3-3</i>		This study
<i>Nigella damascena</i>	<i>NidaAP3-1</i>		This study
	<i>NidaAP3-2</i>		This study
	<i>NidaAP3-3</i>		This study
<i>Nigella sativa</i>	<i>NisaAP3-2</i>	HQ694795	2
	<i>NisaAP3-3</i>	HQ694794	2

Table S4. Cont.

Taxon and species*	Gene name*	Accession no.†	Source
<i>Ranunculus ficaria</i>	<i>RafiAP3-1</i>	AF052854	5
	<i>RafiAP3-2</i>	AF130870	6
	<i>RafiAP3-3</i>	AY162883	3
<i>Ranunculus japonicus</i>	<i>RajaAP3-1</i>		This study
	<i>RajaAP3-2</i>		This study
	<i>RajaAP3-3</i>		This study
<i>Souliea vaginata</i>	<i>SovaAP3-1</i>		This study
	<i>SovaAP3-2</i>		This study
	<i>SovaAP3-3</i>		This study
<i>Thalictrum dioicum</i>	<i>ThdiAP3-1</i>	AY867875	7
	<i>ThdiAP3-2a</i>	AY867876	7
	<i>ThdiAP3-2b</i>	AY867877	7
<i>Thalictrum petaloideum</i>	<i>ThpeAP3-1</i>		This study
	<i>ThpeAP3-2b</i>		This study
<i>Trautvetteria caroliniensis</i>	<i>TrcaAP3</i>	AY162904	3
<i>Trollius laxus</i>	<i>TrlaAP3-1_1</i>	AY162890	3
	<i>TrlaAP3-2_1</i>	AY162892	3
	<i>TrlaAP3-3_1</i>	AY162894	3
<i>Xanthorhiza simplicissima</i>	<i>XasiAP3-2</i>	EU481797	1
	<i>XasiAP3-3</i>	EU481796	1
Berberidaceae			
<i>Berberis gilgiana</i>	<i>BegiAP3-1</i>	AY162857	3
	<i>BegiAP3-2_1</i>	AY162858	3
<i>Epimedium grandiflorum</i>	<i>EpgrAP3-1</i>	EU481793	1
	<i>EpgrAP3-3</i>	EU481794	1
<i>Jeffersonia diphylla</i>	<i>JediAP3-1</i>	EU481791	1
Menispermaceae			
<i>Cocculus trilobus</i>	<i>CotrAP3-1</i>	HQ694788	2
	<i>CotrAP3-2</i>	HQ694789	2
	<i>CotrAP3-3</i>	HQ694790	2
<i>Menispermum dauricum</i>	<i>MedaAP3-1</i>	EU481786	1
	<i>MedaAP3-2</i>	EU481784	1
	<i>MedaAP3-3_1</i>	EU481783	1
Circaeasteraceae			
<i>Circaeaster agrestis</i>	<i>CiarAP3a</i>	HQ647369	2
	<i>CiarAP3b</i>	HQ647370	2
<i>Kingdonia uniflora</i>	<i>KiunAP3a</i>	HQ647371	2
	<i>KiunAP3b</i>	HQ647372	2
Lardizabalaceae			
<i>Akebia quinata</i>	<i>AkquAP3-1_1</i>	AY162835	3
	<i>AkquAP3-2_1</i>	AY162839	3
<i>Akebia trifoliata</i>	<i>AktrAP3-1</i>	AY627630	8
	<i>AktrAP3-2</i>	AY627631	8
	<i>AktrAP3-3</i>	DQ303124	8
<i>Decaisnea insignis</i>	<i>DeinAP3-1</i>	JQ806400	9
	<i>DeinAP3-2</i>	JQ806401	9
<i>Holboellia coriacea</i>	<i>HocoAP3-1</i>	EU481789	1
	<i>HocoAP3-2</i>	EU481788	1
<i>Holboellia grandiflora</i>	<i>HogrAP3</i>	JQ806404	9
<i>Sinofranchetia chinensis</i>	<i>SichAP3-1</i>	JQ806394	9
	<i>SichAP3-2</i>	JQ806395	9
	<i>SichAP3-3</i>	JQ806396	9
Papaveraceae			
<i>Eschscholzia californica</i>	<i>EscaDEF1</i>	EF378697	10
	<i>EscaDEF2</i>	EF378698	10
<i>Papaver nudicaule</i>	<i>PanuAP3-1</i>	AF052873	5
	<i>PanuAP3-2</i>	AF052874	5
<i>Papaver somniferum</i>	<i>PasoAP3-1</i>	EF071993	—
	<i>PasoAP3-2</i>	EF071992	—
<i>Sanguinaria canadensis</i>	<i>SacaAP3</i>	AF130868	6
<i>Dicentra eximia</i>	<i>DiexAP3</i>	AF052875	5
Eupteleaceae			

Table S4. Cont.

Taxon and species*	Gene name*	Accession no.†	Source
<i>Euptelea polyandra</i>	<i>EupoAP3</i>	EU481781	1
Trochodendraceae			
<i>Trochodendron aralioides</i>	<i>TrarAP3</i>	DQ453774	11
Sabiaceae			
<i>Meliosma dilleniifolia</i>	<i>MediAP3-1</i>	AY436709	12
Buxaceae			
<i>Pachysandra procumbens</i>	<i>Paprap3-1</i>	DQ479360	11
Magnoliaceae			
<i>Magnolia praecocissima</i>	<i>MpMADS7</i>	AB050649	—
Chloranthaceae			
<i>Chloranthus spicatus</i>	<i>ChspAP3</i>	AF230701	13
Aristolochiaceae			
<i>Aristolochia eriantha</i>	<i>ArerAP3</i>	AY436714	12

*Species sampled and genes cloned in this study are highlighted in bold.

†The sequences were retrieved from National Center for Biotechnology Information (NCBI).

‡The sequences were retrieved from the *Aquilegia* genome database (www.phytozome.net).

- Rasmussen DA, Kramer EM, Zimmer EA (2009) One size fits all? Molecular evidence for a commonly inherited petal identity program in Ranunculales. *Am J Bot* 96(1):96–109.
- Sharma B, Guo C, Kong H, Kramer EM (2011) Petal-specific subfunctionalization of an *APETALA3* paralog in the Ranunculales and its implications for petal evolution. *New Phytol* 191(3):870–883.
- Kramer EM, Di Stilio VS, Jaramillo MA (2003) Complex patterns of gene duplication in the *APETALA3* and *PISTILLATA* lineages of the Ranunculaceae. *Int J Plant Sci* 164:1–11.
- Kramer EM, et al. (2007) Elaboration of B gene function to include the identity of novel floral organs in the lower eudicot *Aquilegia*. *Plant Cell* 19(3):750–766.
- Kramer EM, Dorit RL, Irish VF (1998) Molecular evolution of genes controlling petal and stamen development: Duplication and divergence within the *APETALA3* and *PISTILLATA* MADS-box gene lineages. *Genetics* 149(2):765–783.
- Kramer EM, Irish VF (1999) Evolution of genetic mechanisms controlling petal development. *Nature* 399(6732):144–148.
- Di Stilio VS, Kramer EM, Baum DA (2005) Floral MADS-box genes and homeotic gender dimorphism in *Thalictrum dioicum* (Ranunculaceae) — A new model for the study of dioecy. *Plant J* 41(5):755–766.
- Shan H, et al. (2006) Conservation and divergence of candidate class B genes in *Akebia trifoliata* (Lardizabalaceae). *Dev Genes Evol* 216(12):785–795.
- Hu J, Zhang J, Shan H, Chen Z (2012) Expression of floral MADS-box genes in *Sinofranchetia chinensis* (Lardizabalaceae): Implications for the nature of the nectar leaves. *Ann Bot (Lond)* 110(1):57–69.
- Zahn LM, et al. (2005) The evolution of the *SEPALLATA* subfamily of MADS-box genes: A preangiosperm origin with multiple duplications throughout angiosperm history. *Genetics* 169(4):2209–2223.
- Kramer EM, Su HJ, Wu CC, Hu JM (2006) A simplified explanation for the frameshift mutation that created a novel C-terminal motif in the *APETALA3* gene lineage. *BMC Evol Biol* 6:30.
- Stellari GM, Jaramillo MA, Kramer EM (2004) Evolution of the *APETALA3* and *PISTILLATA* lineages of MADS-box-containing genes in the basal angiosperms. *Mol Biol Evol* 21(3):506–519.
- Kramer EM, Irish VF (2000) Evolution of the petal and stamen developmental programs: Evidence from comparative studies of the basal angiosperms. *Int J Plant Sci* 161:S29–S40.