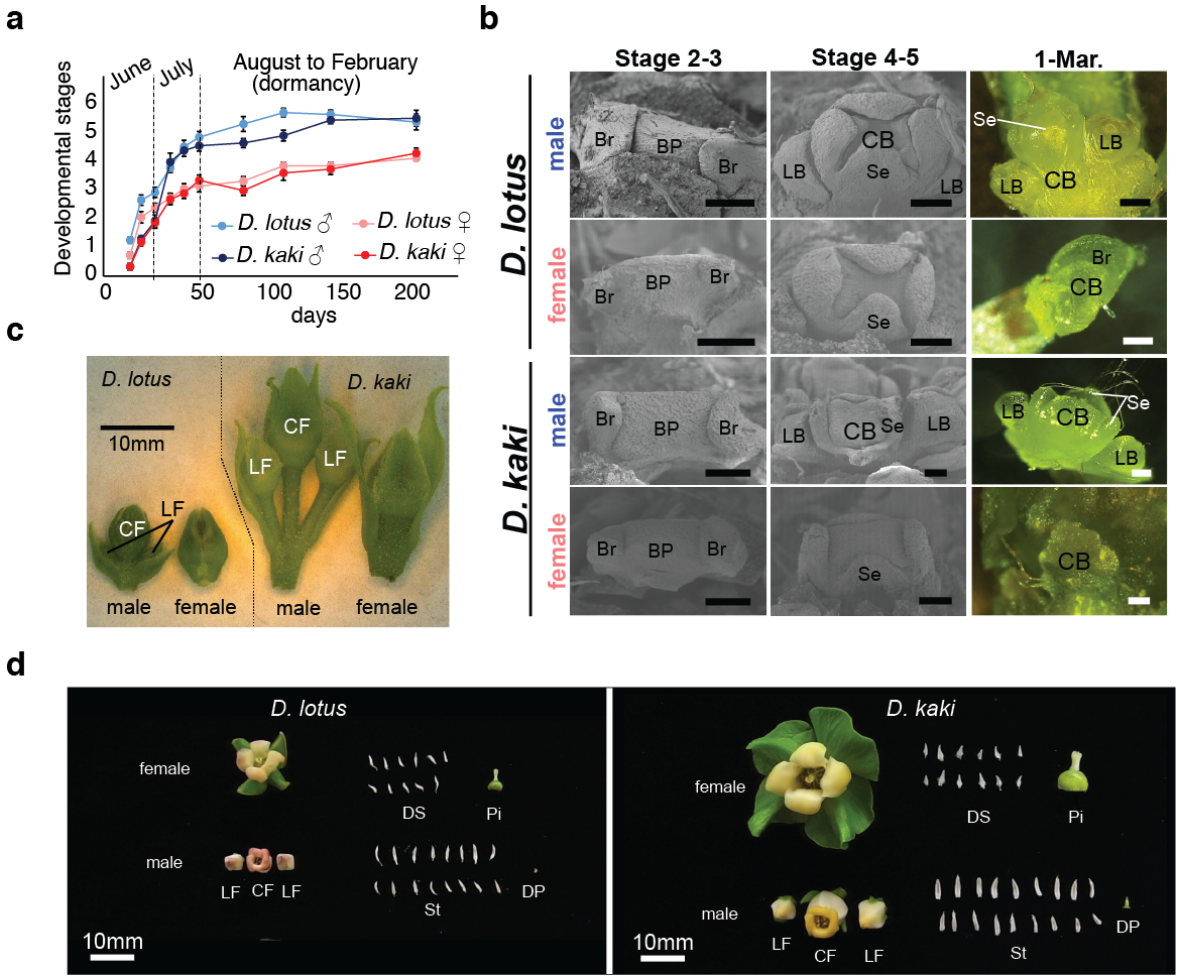
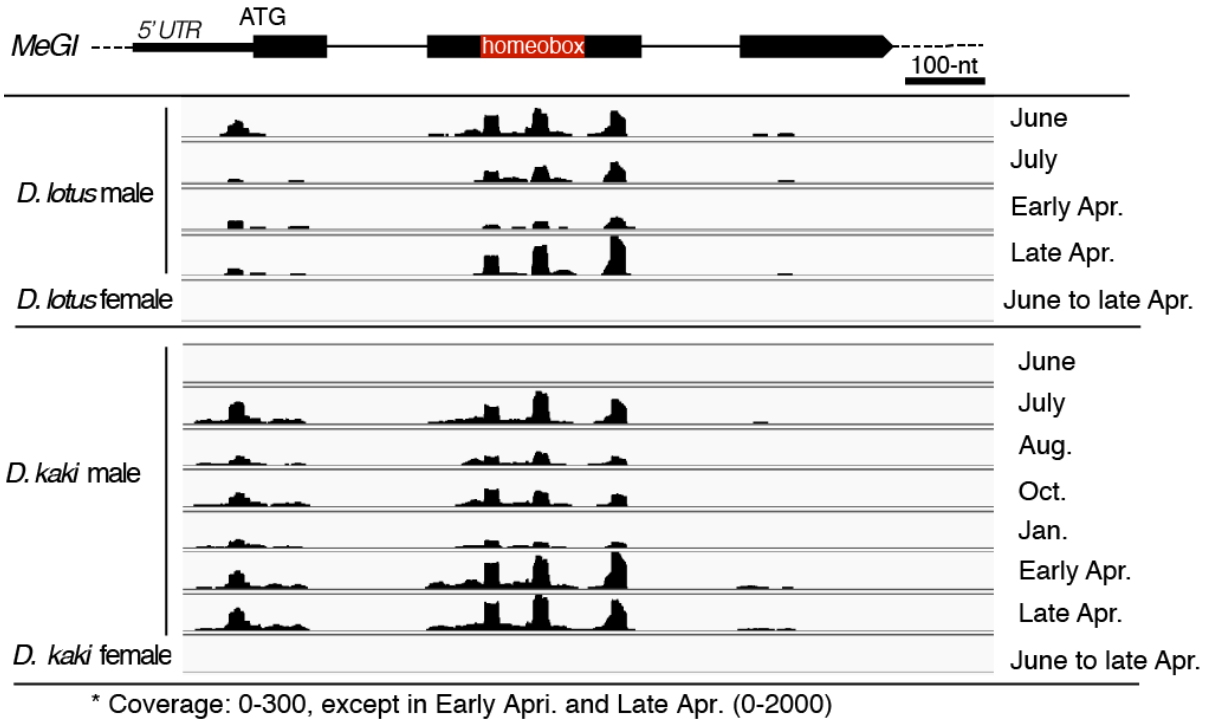
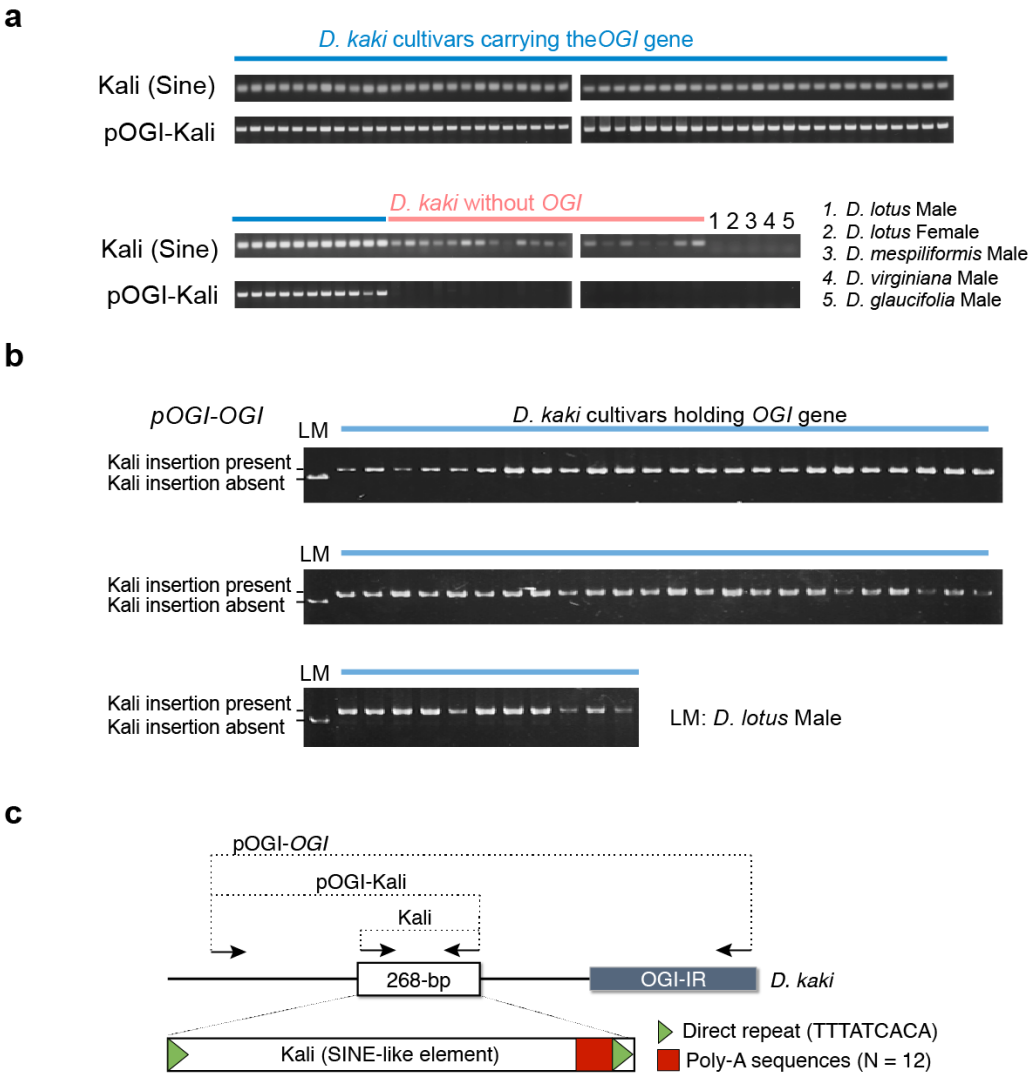


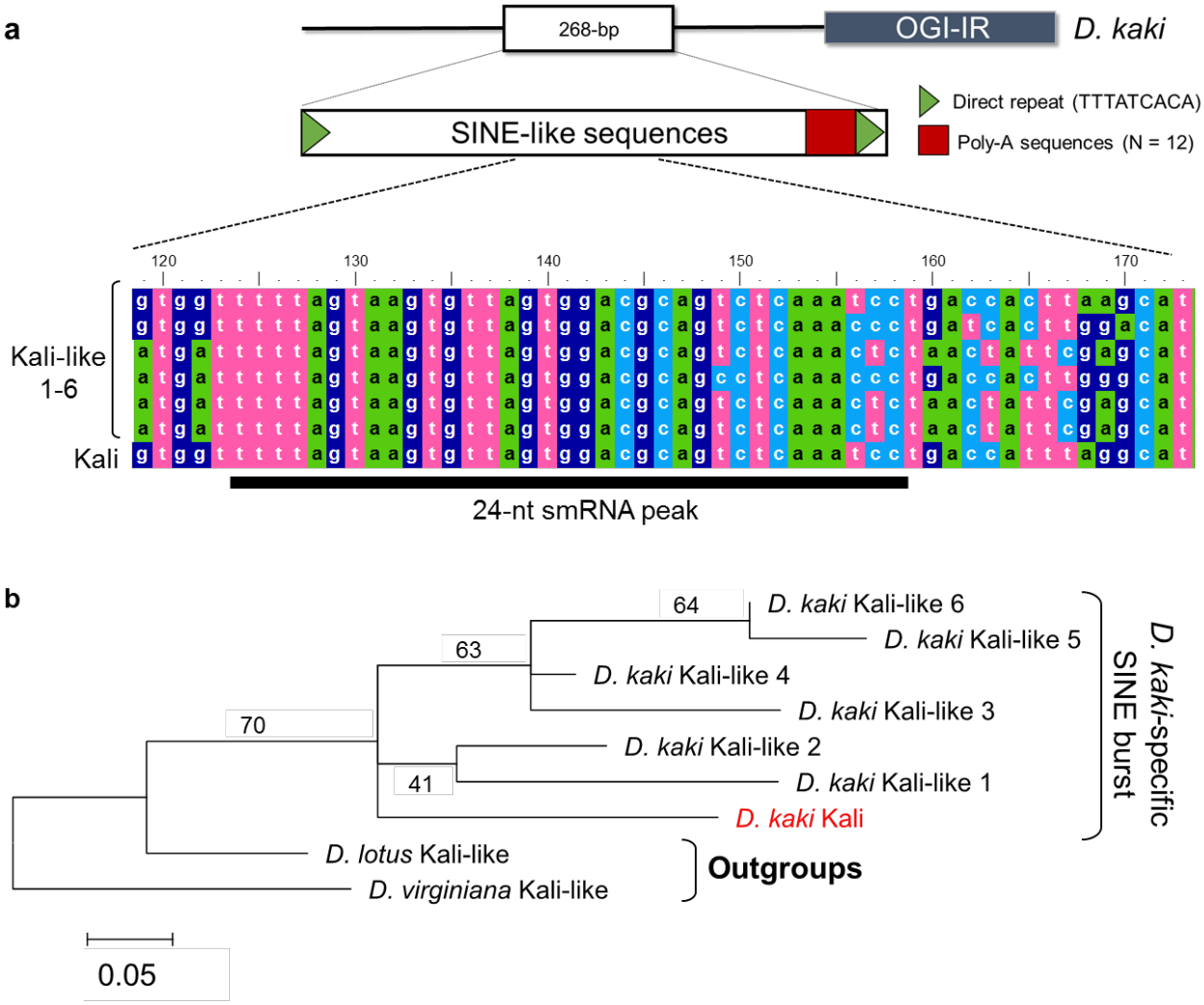
Supplemental Figure 1. Comparison of the developmental stages of *D. lotus* and *D. kaki* flowers. a, Developmental stages of male and female flower primordia in *D. lotus* and *D. kaki.* Specific developmental stages were assigned a number based on a previous report (Yonemori et al., 1993). The mean stage numbers are shown for male and female *D. kaki* and *D. lotus* flower primordia (*N* = 10-15), and standard errors are indicated. After July 1st (30 days), most male primordia had reached stages 4-5 and exhibited differentiated sepals before dormancy was established. In contrast, at that time, female primordia had on average only reached stage 3. **b,** Observation of the male and female primordia under SEM and dissecting microscopes. In both *D. lotus* and *D. kaki*, male primordia were trifurcated and contained a central bud (CB) and two lateral buds (LB), corresponding to stages 4-5, before entering dormancy. When emerging from dormancy, in March of the next year, male flowers exhibited differentiated sepals (Se), while female flowers were at stage 3-4 (see panel **a**), with no distinct flower organs. Br: bract, BP: bud primordia. Bars indicate 50 μm. **c,** Male and female *D. lotus* and *D. kaki* unopened flowers. CF: central flower, LF: lateral flower. **d,** Male and female flower organs in *D. lotus* and *D. kaki*. Overall, *D. lotus* and *D. kaki* exhibited similar timing and morphological differences between male and female flowers across developmental stages. DS: defective stamens (no pollen), St: stamens, Pi: pistil, DP: defective pistil.



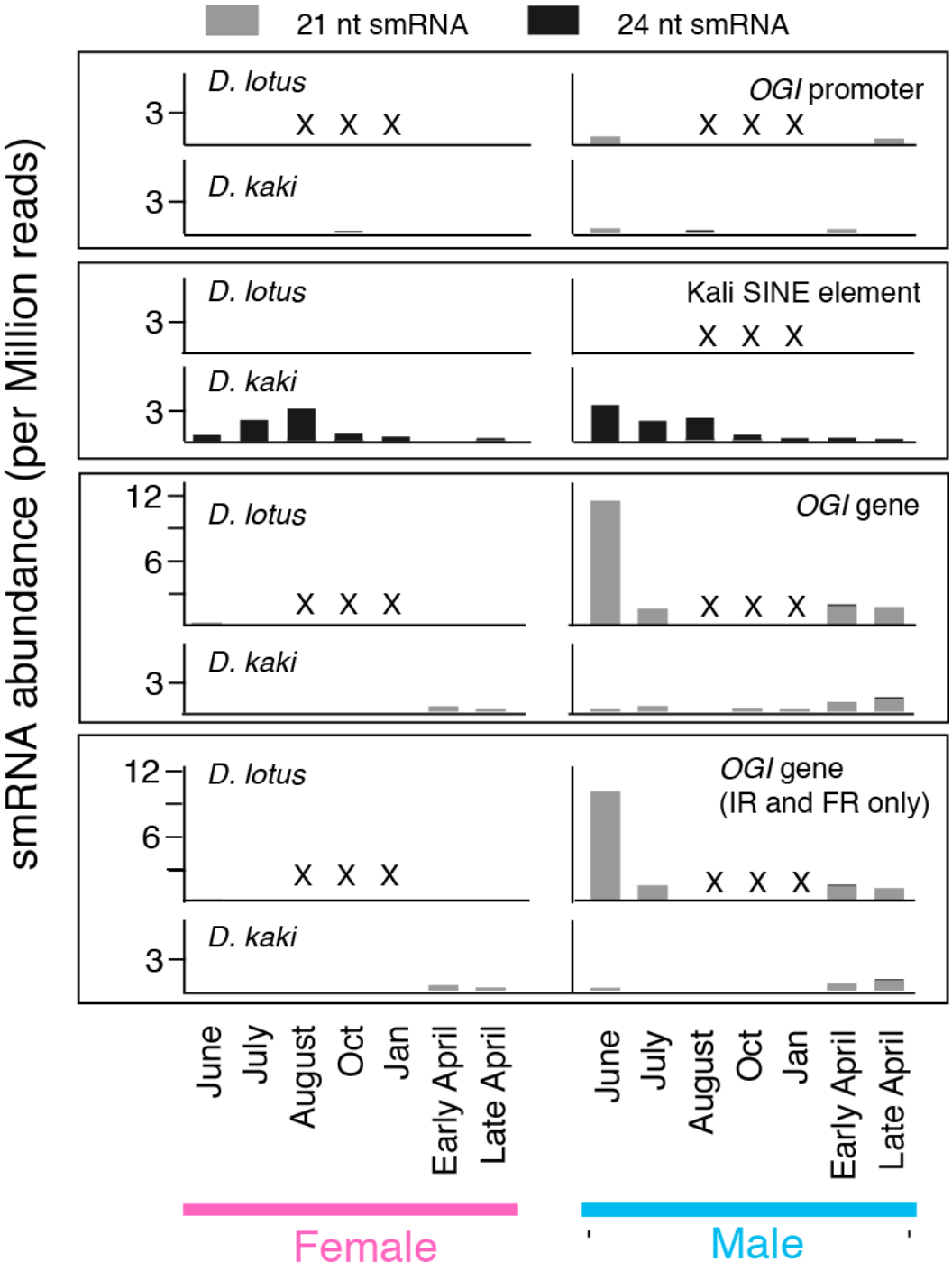
Supplemental Figure 2. Pattern of smRNA accumulation on the *MeGI* transcript.
smMeGI distribution in male and female buds/flowers in dioecious *D. lotus* and monoecious *D. kaki* throughout the year.



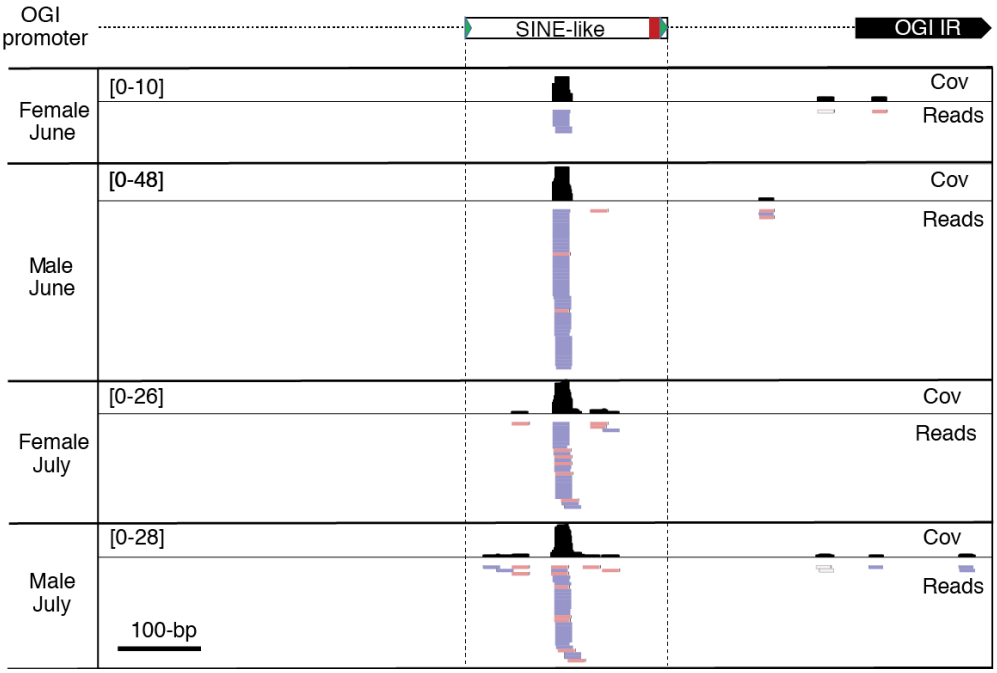
Supplemental Figure 3. Conservation of *D. kaki*-specific SINE insertions in the *OGI* 5' region. a-b, PCR analysis of the Kali SINE-like sequences in the *OGI* 5' promoter region. **a,** The Kali-in primer set (see panel **c**) was designed to amplify sequences within the SINE insertion. Kali amplicons were detected in a wide variety of *D. kaki* cultivars carrying the *OGI* gene(s) (blue lines), as well as in cultivars that do not carry the *OGI* sequences (pink line), but not in other *Diospyros* species. This suggested that this SINE-like sequence is specific to *D. kaki*. The pOGI-Kali primer set (see panel **c**) was designed to specifically detect the SINE sequence copy that flanks the *OGI* gene. Among the 172 cultivars tested, all 59 cultivars carrying *OGI* also carried the Kali insertion in the promoter region. This *OGI*-specific SINE insertion was not detected in male individuals of other dioecious *Diospyros* species (nos. 1, 3, 4, and 5). **b,** The pOGI-*OGI* primer set (see panel **c**) amplifies the *OGI* promoter across the Kali insertion (when present). Size of the PCR product thus indicates the presence or absence of the Kali insertion. All cultivars tested contained the Kali insertion, demonstrating that it is conserved in all alleles in *D. kaki*. **c,** Schematic structure of the *OGI* promoter region and the Kali-in and *OGI*-Kali primer sets.



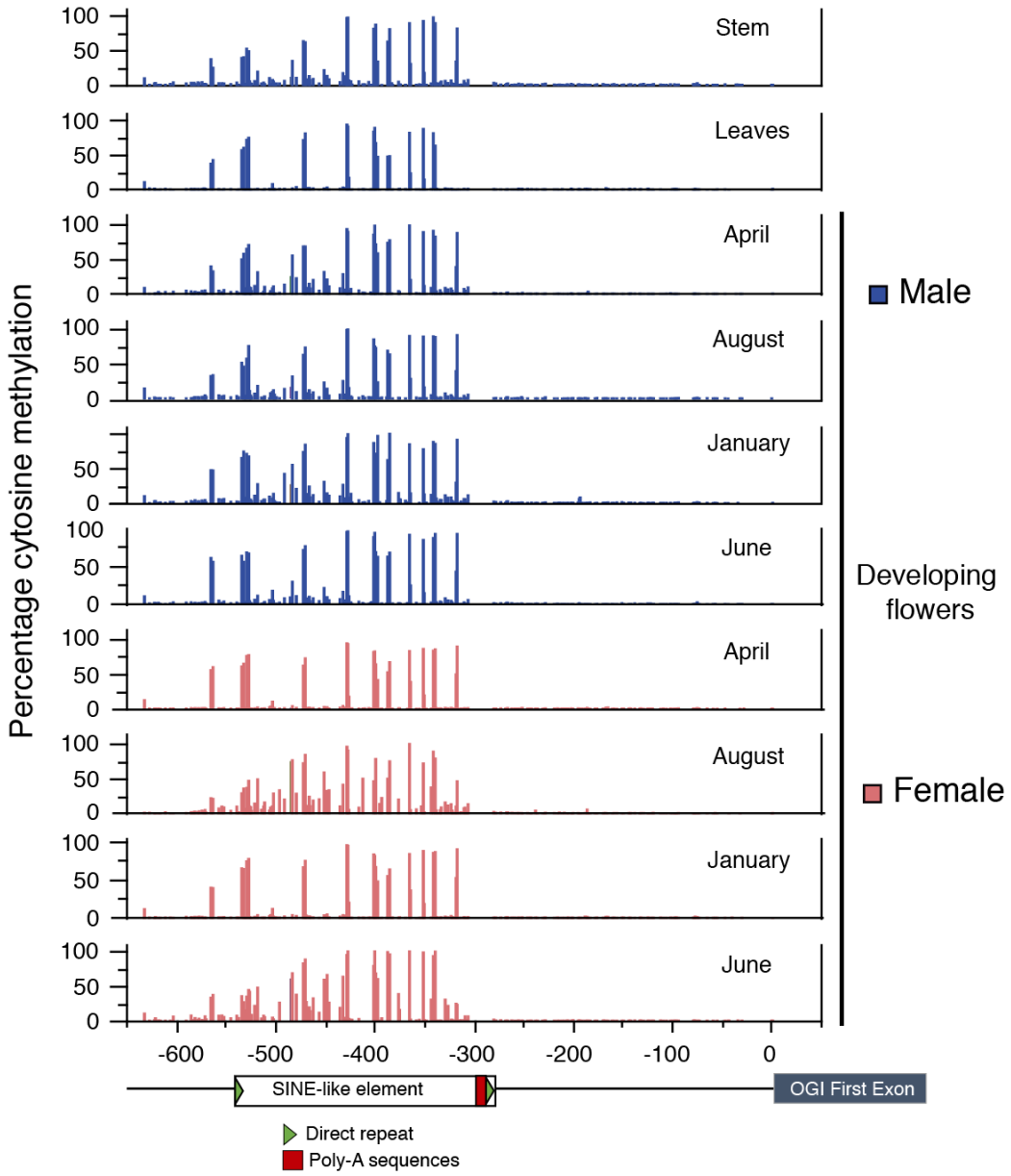
Supplemental Figure 4. Analysis of Kali-like sequences in the *Diospyros* genome. a. Partial alignment of the Kali and Kali-like sequences from *D. kaki*. The regions including the 24-nt smRNA sequence were aligned to characterize variation among the Kali-like sequences. Kali was identical to Kali-like 1 across the smRNA sequence. **b.** Phylogenetic analysis of the divergence of Kali and Kali-like SINEs. Kali and 6 Kali-like sequences were derived from *D. kaki* SINE amplified sequences, which were not observed in the genomes of *D. lotus* and *D. virginiana*, the closest relatives of *D. kaki*. Bootstrap values are shown as percentages on the branches.



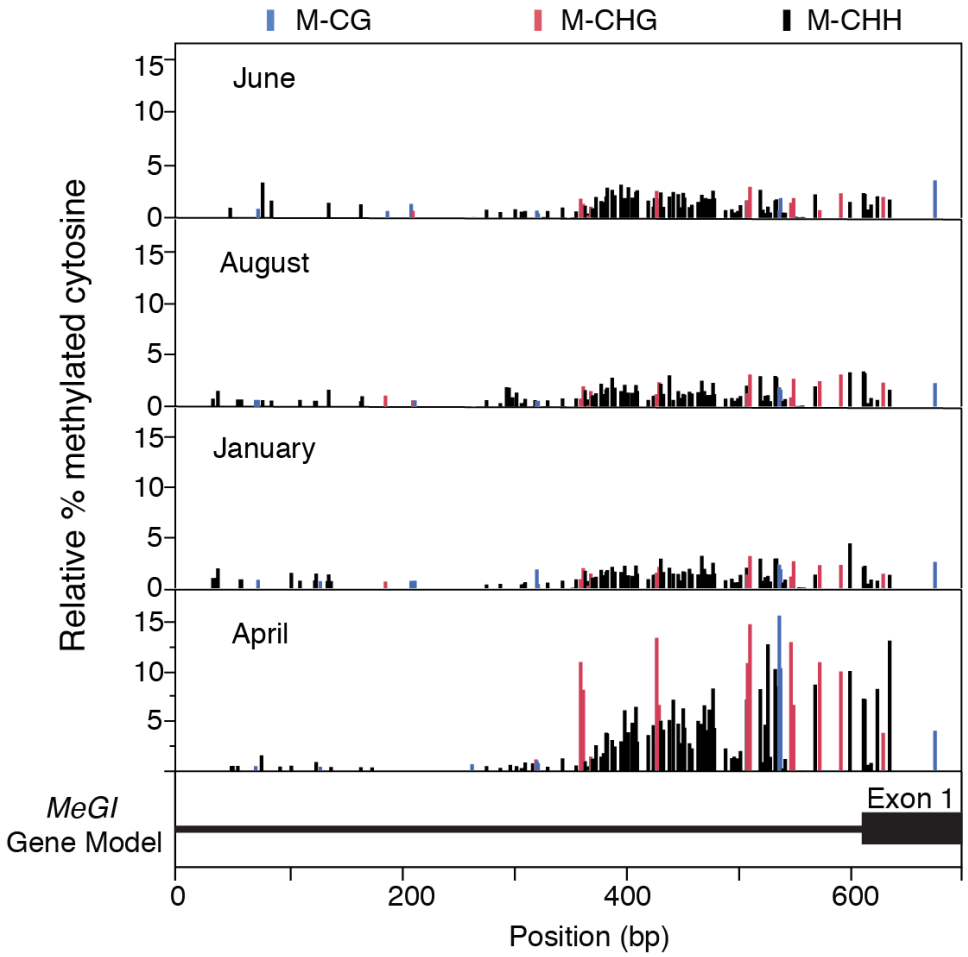
Supplemental Figure 5. Small RNAs targeting the *OGI* gene and promoter elements. Abundance of 21 nt (gray) and 24 nt (black) smRNA targeting *OGI* during primordia formation, bud dormancy, and flower development, in developing buds and branches of monoecious *D. kaki* and dioecious *D. lotus.* Values are expressed in reads per million. 21 nt smRNA and 24 nt smRNA levels are stacked on top of each other for each data point. The different sequences analyzed are: promoter (a total of 500 bps immediately upstream of the start codon but excluding the Kali element), Kali SINE element (the 256-bp SINE element), *OGI* gene (genomic sequence including introns), and finally the *OGI* gene (IR and FR only). An "X" indicates a time-point for which data are not available".



Supplemental Figure 6. 24-nt small RNA accumulation on the Kali SINE-like insertion in the *OGI* promoter. The structure of the *OGI* promoter region is shown on top. For each sample type, the coverage track is shown in black above the smRNA mapping tracks. All smRNA mapped to the Kali SINE-like region are 24-nt long. Mapped reads are shown in different colors depending on their mapping quality, with unambiguously mapped reads shown in pink (forward mapped reads) or blue (reversely mapped reads) and ambiguously mapped reads shown in gray.

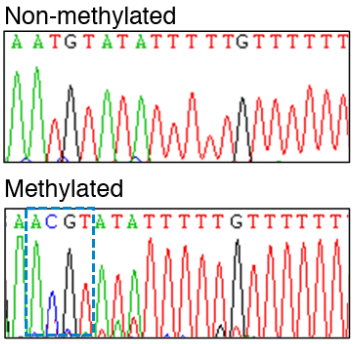


Supplemental Figure 7. DNA methylation across the *OGI* promoter. Cytosine methylation levels across the *OGI* promoter in a variety of tissue types from hexaploid *D. kaki* cultivar Taishu. Each bar represents one cytosine residue in either the sense or antisense strand. The position of the SINE element relative to the start codon of the *OGI* pseudo-gene is represented at the bottom. Young stems and leaves from male branches (harvested in May), as well as developing flowers from male and female branches at various developmental stages were analyzed.

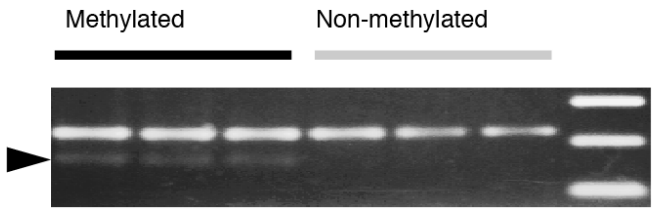


Supplemental Figure 8. Seasonal DNA methylation on the *MeGI* promoter in male buds/flowers. Variation in DNA methylation levels across the *MeGI* promoter region in developing male flowers of monoecious *D. kaki*. Different colors represent the different sequence contexts (CH, CHG, and CHH, are indicated in blue, red, and black, respectively). The gene model is shown at the bottom. Methylation data values at each position were normalized based on the control gene *MatK*.

a



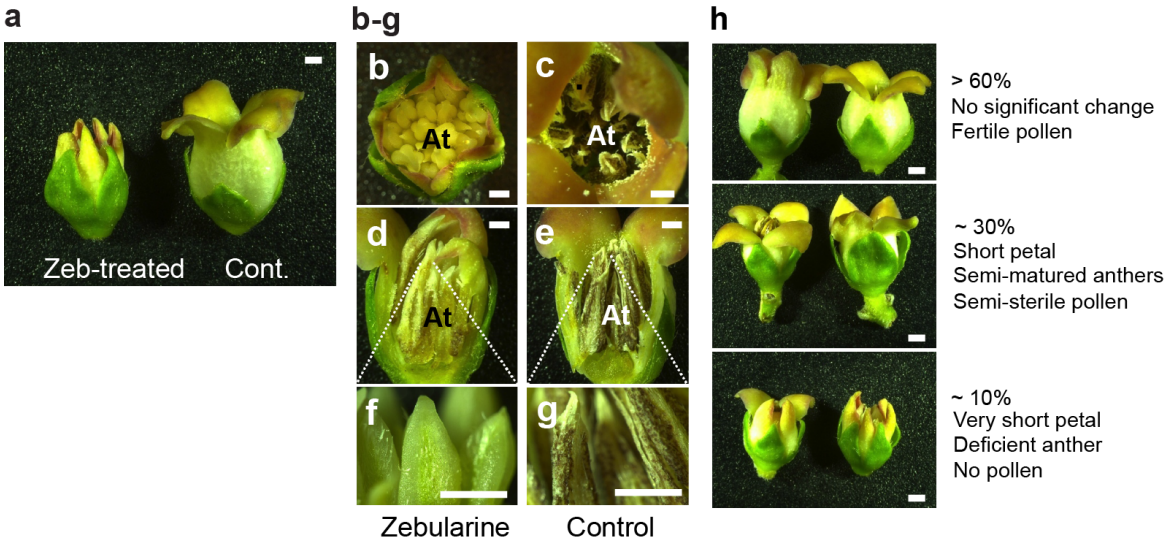
b



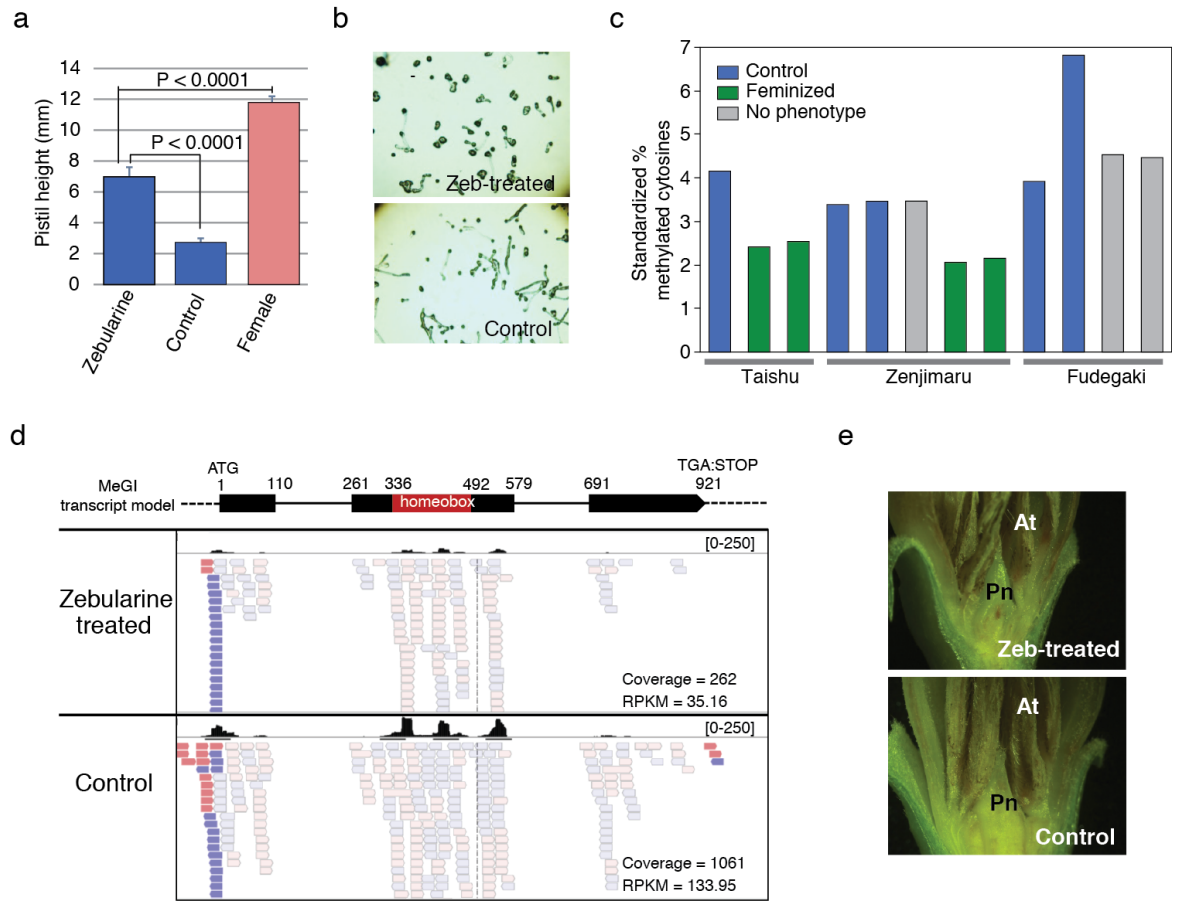
c

	Bud position						
	1	2	3	4	5	6	7
Methylated	4	4	2	1	1	0	0
Non-methylated	5	5	4	5	6	7	12
Methylation rate	0.444	0.444	0.333	0.167	0.143	0.000	0.000

Supplemental Figure 9. DNA methylation of the *MeGI* promoter in single developing buds. **a**: Bisulfite-treated sequences of methylated and non-methylated portions of the *MeGI* promoter. Methylation of a cytosine residue located 26-bp upstream from the start codon results in the gain of an *Hpy*CH4 IV restriction site (ACGT, dotted blue box). This was converted into a CAPs marker and used as a proxy to investigate cytosine methylation in individual developing buds from cvs. Taishu and Zenjimaru. **b**: PCR product amplified from bisulfite-treated bud DNA and digested with *Hpy*CH4 IV. Methylated amplicons were digested, while unmethylated amplicons remained intact, as indicated by the arrow. **c**: Number of buds exhibiting cytosine methylation, depending on its position on the female parental branch (see * in Figure 3).



Supplemental Figure 10. Phenotypic effects of zebularine treatment on male *D. lotus* flowers. a, Representative phenotype of zebularine-treated and control *D. lotus* male flowers. On average, petals were smaller after zebularine treatment. **b-g,** Comparison of the anthers in zebularine-treated (b, d, and f) and control (c, e, and g) *D. lotus* male flowers. In the zebularine-treated flowers, stamens did not fully mature (d and f), and could not produce pollen grains. On the other hand, stamens in the control flowers turned brown and fully matured to produce normal pollen. **h,** Distribution of the phenotypes (see Supplemental Table 3 for actual numbers). Over 60% of the zebularine-treated flowers (top) showed no distinct morphological change and produced fertile pollen. Approximately 30% of them exhibited semi-severe phenotypes (middle), where pollen grains were produced normally, but exhibited a significant reduction in germination rate ($P < 0.01$, in Student's T-test) compared to the control pollen grains. The remaining 10% of the zebularine-treated flowers exhibited strong phenotypes and did not produce any pollen grains. The severe phenotypes, i.e., shorter petals and defective anthers, are consistent with the characteristics of Arabidopsis plants transformed by CaMV35S-*MeGI* (Akagi et al. 2014a). Bars indicate 1 mm in all panels.



Supplemental Figure 11. Effect of zebularine treatment on male *D. kaki* flowers. a-c, Effect of zebularine treatment on cultivar Zenjimaru. **a-b,** Effect of zebularine treatment on pistil length and pollen tube growth. **a,** Comparison of pistil length in male zebularine-treated and control flowers, as well as female flowers. Pistils from zebularine-treated flowers were significantly longer than those from the control male flowers ($P < 0.0001$, Student's T-test, $n = 10$, standard errors are shown) but shorter than those from female flowers ($P < 0.0001$, Student's T-test, $n = 10$). **b,** Pollen tube growth in zebularine-treated and control male flowers. The zebularine-treated flowers showed immature stamens and a significant reduction in pollen viability (Fig. 5B). **c,** Percentage of methylated cytosine residue in the promoter region of the *MeGI* gene of zebularine-treated (green and gray bars) or control (blue bars) flowers in three different cultivars (Zenjimaru, Taishu and Fudegaki). The percentage of methylated cytosine at all cytosine positions was averaged over *ca.* 400-bp region including 250-bps upstream of the start codon and 150-bp of the first exon. For each cultivar, zebularine-treated samples were categorized as feminized or WT depending on their phenotypic characteristics. Methylation data values at each position were normalized based on the control gene *MatK*. **d,** *smMeGI* population from zebularine-treated and control developing flowers. The exon/intron model for the *MeGI* transcript is shown on top. Below the model are sm*MeGI* smRNA-Seq reads from zebularine-treated and control flowers. Coverage tracks and some of the mapped reads are shown for each sample. In the zebularine-treated flowers, the expression level of *smMeGI* was reduced to approximately 25% of the control, but the pattern of smRNA accumulation across the *MeGI* transcript was conserved. Numbers in brackets ([0-250]) indicate the scale for the coverage tracks shown above (in black, indicating the distribution of the mapped reads). **e,** Zebularine-treated cv. Fudegaki male. In cv. Fudegaki, which carries the *OGI* gene and is monoecious, zebularine treatment has no morphological effect on the gynoecia or the androecia. Anthers (At) in both the zebularine-treated and the control flowers exhibited normal ability to produce fertile pollen, and pistinodes (Pn) did not bear seed.

Supplemental Table 1: Small-*MeGI* expression levels in male buds/flowers of cv. Taishu throughout the year. The normalized RPKM values in each length are given. Except during the flower development stages (early-Apr and late Apr), 21-nt *smMeGI* were predominant in male buds.

	small-RNA levels on *MeGI* (RPKM)			
	21-nt	**22-nt**	**23-nt**	**24-nt**
Jun	0.7	0.2	0.1	0.3
Jul	73.8	7.9	2.5	3.0
Aug	63.2	6.6	0.7	2.0
Oct	36.9	3.3	0.5	1.1
Jan	26.7	2.8	0.7	0.7
early Apr	516.5	153.6	32.4	46.8
late Apr	303.1	60.2	12.9	24.5

Supplemental Table 2: Multiple discriminant analysis (MDA) of the pattern of bud/flower sexuality based on biological and environmental variables. A model for flower sexuality was built based on data from eight cultivars (see Methods). Posterior probabilities obtained in representative conditions based on (i) sexuality of the parental branch, (ii) parental branch length, and (iii) bud position are indicated. For each branch, position is determined relative to the base of the branch, with the apical buds bearing the highest number.

Gender of parent branch	Length of parent branch (cm)	bud position	Posterior probability			Note
			Female	Male	no flower	
Female	40	8	0.931	0.047	0.022	Used as female bud for all analyses presented in this report
Female	40	7	0.925	0.048	0.027	
Male	12	3	0.044	0.947	0.009	Used as male bud for all analyses presented in this report
Male	12	2	0.024	0.963	0.013	
Male	12	1	0.013	0.969	0.018	

Supplemental Table 3: DNA methylation status of the *MeGI* promoter throughout the year. Comparison of CG-CHG-CHH DNA methylation percentages on the *MeGI* promoter region. Mean methylation percentage were calculated across the 284 bps directly upstream of the *MeGI* start codon. Developing flowers sampled throughout the year were analyzed.

	Male				Female			
	Jun	Aug	Jan	Apr	Jun	Aug	Jan	Apr
CG								
SS	0.38	0.39	0.68	4.05	0.07	0.22	0.14	0.20
AS	1.41	1.20	1.58	11.03	0.16	0.21	0.17	0.19
CHG								
SS	0.15	0.24	0.43	2.21	0.12	0.09	0.09	0.22
AS	1.90	1.83	2.82	9.52	0.15	0.29	0.20	0.23
CHH								
SS	0.39	0.40	0.54	0.90	0.19	0.20	0.23	0.23
AS	2.12	1.54	3.30	3.46	0.21	0.33	0.20	0.23

SS: Sense strand, AS: Antisense strand.

Supplemental Table 4: Flower phenotypes after zebularine treatment in *D. kaki* and *D. lotus*. a, Number of semi-feminized and male zebularine-treated and control flowers in three *D. kaki* cultivars. In cvs. Zenjimaru and Taishu, semi-feminized flowers were also observed in control samples, although at a low rate. Here, the semi-feminized flowers were all similar in appearance. **b,** Number of flowers for which zebularine treatment resulted in strong, intermediate, and no substantial effect in diploid *D. lotus*. Photographs of each of the phenotypic classes are presented in Supplemental Figure 10.

a

Cultivar (genotype)	year		semi-feminized (fruited)	male
cv. Zenjimaru (XXXXXY)	2015	Zeb-treated	28 (0)	3
		Control	1 (1)	59
cv. Fudegaki (XXXXXY)	2015	Zeb-treated	0	45
		Control	0	52
cv. Zenjimaru (XXXXXY)	2016	Zeb-treated	31 (0)	9
		Control	1 (0)	28
cv. Fudegaki (XXXXXY)	2016	Zeb-treated	0	25
		Control	0	19
cv. Taishu (XXXXYY)	2016	Zeb-treated	20 (4)	12
		Control	2 (2)	24

b

Cultivar (genotype)		Strong	Intermediate	No effect
cv. Kunsenshi-male (XY)	Zeb-treated	9	28	63
	Control	0	0	100
cv. Kunsenshi-female (XX)	Zeb-treated	0	0	105
	Control	0	0	105

Supplemental Table 5: List of plant materials

species	cultivar/accession	sexuality	sampling location	annotation
Diospyros lotus	Kunsenshi Male	male	Kyoto Univ, Kyoto, Japan	male parent of the KK F_1 pop
	Kunsenshi Female	female	Kyoto Univ, Kyoto, Japan	female parent of the KK F_1 pop
	KK1-L1	male	Kyoto Univ, Kyoto, Japan	the KK F1 population
	KK1-L8	male	Kyoto Univ, Kyoto, Japan	the KK F1 population
	KK1-L7	female	Kyoto Univ, Kyoto, Japan	the KK F1 population
	KK1-L18	female	Kyoto Univ, Kyoto, Japan	the KK F1 population
	KK1-L20	female	Kyoto Univ, Kyoto, Japan	the KK F1 population
D. glaucifolia	Male no. 10	male	Kyoto Univ, Kyoto, Japan	Alternatively, *Diospyros japonica*
D. virginiana	DDIO 69 0003A	male	USDA/ARS, Davis, CA, USA	putative tetraploid or hexaploid
D. mespiliformis	MIA3483	male	USDA/ARS, Miami, FL, USA	
	MIA1079	male	USDA/ARS, Miami, FL, USA	
D. kaki[a]	Taishu	monoecious[b]	Kyoto Univ, Kyoto, Japan	MDA, EA, MA, ZT, OP[c]
	Zenjimaru	monoecious	Kyoto Univ, Kyoto, Japan	MDA, EA, ZT, OP
	Tohachi	monoecious	Kyoto Univ, Kyoto, Japan	MDA, EA, OP
	Kakiyamagaki	monoecious	Kyoto Univ, Kyoto, Japan	MDA, EA, OP
	Okugosho	monoecious	Kyoto Univ, Kyoto, Japan	MDA, OP
	Amayotsumizo	monoecious	Kyoto Univ, Kyoto, Japan	MDA, OP
	Fudegaki	monoecious	Kyoto Univ, Kyoto, Japan	MDA, ZT, OP
	Iwasedo	monoecious	Kyoto Univ, Kyoto, Japan	MDA, OP
	Hazegosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Egosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Meotogaki	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Fujiwaragosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Taiwanshoshi	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Hanagosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Kanshu	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Seihakuji	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Yamagaki	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Kikumanju	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Nishimurawase	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Ibogaki	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Shogatsu	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Akadu	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Saburoza	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Shojo	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Kyara	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Tenjingosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Issaigaki	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Shirotodamashi	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Yotsumizo	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Yamatogosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Mushirodagosho	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Shozaemon	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Cal.Fuyu	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Muraya	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Emon	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Toyoka	monoecious	Kyoto Univ, Kyoto, Japan	OP
	Nagara	female	Kyoto Univ, Kyoto, Japan	OP
	Kunitomi	female	Kyoto Univ, Kyoto, Japan	OP
	Deshimaru	female	Kyoto Univ, Kyoto, Japan	OP

Shoro	female	Kyoto Univ, Kyoto, Japan	OP
Tyagone	female	Kyoto Univ, Kyoto, Japan	OP
Yoshino	female	Kyoto Univ, Kyoto, Japan	OP
Yashima	female	Kyoto Univ, Kyoto, Japan	OP
Nitari	female	Kyoto Univ, Kyoto, Japan	OP
Kawagone	female	Kyoto Univ, Kyoto, Japan	OP
Atagobo	female	Kyoto Univ, Kyoto, Japan	OP
Sanenashi	female	Kyoto Univ, Kyoto, Japan	OP
Nanshi	female	Kyoto Univ, Kyoto, Japan	OP
Beniemon	female	Kyoto Univ, Kyoto, Japan	OP
Ogosho	female	Kyoto Univ, Kyoto, Japan	OP
Suruga	female	Kyoto Univ, Kyoto, Japan	OP
Monpei	female	Kyoto Univ, Kyoto, Japan	OP
Beniwase	female	Kyoto Univ, Kyoto, Japan	OP
Oniwa	female	Kyoto Univ, Kyoto, Japan	OP
Shimokitahagakushi	female	Kyoto Univ, Kyoto, Japan	OP
Mikado	female	Kyoto Univ, Kyoto, Japan	OP
Kodagosho	female	Kyoto Univ, Kyoto, Japan	OP
Chichibu-Issaigaki	female	Kyoto Univ, Kyoto, Japan	OP
Saisho	female	Kyoto Univ, Kyoto, Japan	OP
Fuyu	female	Kyoto Univ, Kyoto, Japan	OP
Saijo	female	Kyoto Univ, Kyoto, Japan	OP
Jiro	female	Kyoto Univ, Kyoto, Japan	OP
Aizumishirazu	female	Kyoto Univ, Kyoto, Japan	OP
Mikatanigosho	female	Kyoto Univ, Kyoto, Japan	OP
Atagobo	female	Kyoto Univ, Kyoto, Japan	OP
Shinshu	female	Kyoto Univ, Kyoto, Japan	OP
Soshu	female	Kyoto Univ, Kyoto, Japan	OP
Koshuhyakume	female	Kyoto Univ, Kyoto, Japan	OP
Gionbo	female	Kyoto Univ, Kyoto, Japan	OP
Oyotsumizo	female	Kyoto Univ, Kyoto, Japan	OP
Benigosho	female	Kyoto Univ, Kyoto, Japan	OP
Ichidagaki	female	Kyoto Univ, Kyoto, Japan	OP
Dojohachiya	female	Kyoto Univ, Kyoto, Japan	OP
Obishi	female	Kyoto Univ, Kyoto, Japan	OP
Komino	female	Kyoto Univ, Kyoto, Japan	OP
Amahyakume	female	Kyoto Univ, Kyoto, Japan	OP
Kurokuma	female	Kyoto Univ, Kyoto, Japan	OP
Kuramitsu	female	Kyoto Univ, Kyoto, Japan	OP
Eboshi	female	Kyoto Univ, Kyoto, Japan	OP

[a] *Diospyros kaki* can be hexaploid (6x) or nonaploid (9x), but only hexaploid cultivars were used here.
[b] The gender of the *D. kaki* cultivars was based on information from previous reports. Some monoecious cultivars have been observed to occasionally bear hermaphrodite-like flowers.
[c] For the *D. kaki* trees, the experiments for which each cultivar was used is indicated. MDA: multiple discriminant analysis, EA: expression analysis, MA: methylation analysis, ZT: zebularine treatment, OP: *OGI* promoter PCR test.

Supplemental Table 6: smRNA-Seq sample information.

Library	Species	Cultivar/Accession	Gender	Organ	Time of sampling	Method
smKF_12Jul	*D. lotus*	Kunsenshi Female	female	bud	12-Jul	SR50
smKM_12Jul	*D. lotus*	Kunsenshi Male	male	bud	12-Jul	SR50
smLotKF-Apr26	*D. lotus*	Kunsenshi Female	female	whole flower	26-Apr	SR50
smLotKM-Apr26	*D. lotus*	Kunsenshi Male	male	whole flower	26-Apr	SR50
smRApr1LotFflw	*D. kaki*	Kunsenshi Female	female	whole flower	1-Apr	SR50
smRApr1LotMflw	*D. kaki*	Kunsenshi Male	male	whole flower	1-Apr	SR50
smRApr26TaiFflw	*D. kaki*	Taishu	female	whole flower	26-Apr	SR50
smRApr26TaiMflw	*D. kaki*	Taishu	male	whole flower	26-Apr	SR50
smRApr4TaiFflw	*D. kaki*	Taishu	female	whole flower	4-Apr	SR50
smRApr4TaiMflw	*D. kaki*	Taishu	male	whole flower	4-Apr	SR50
smRJul9TaiF	*D. kaki*	Taishu	female	bud	9-Jul	SR50
smRJul9TaiM	*D. kaki*	Taishu	male	bud	9-Jul	SR50
smRJun9TaiF	*D. kaki*	Taishu	female	bud	9-Jun	SR50
smRJun9TaiM	*D. kaki*	Taishu	male	bud	9-Jun	SR50
smTaiF12Aug	*D. kaki*	Taishu	female	bud	12-Aug	SR50
smTaiF15Jan	*D. kaki*	Taishu	female	bud	15-Jan	SR50
smTaiF15Oct	*D. kaki*	Taishu	female	bud	15-Oct	SR50
smTaiM12Aug	*D. kaki*	Taishu	male	bud	12-Aug	SR50
smTaiM15Jan	*D. kaki*	Taishu	male	bud	15-Jan	SR50
smTaiM15Oct	*D. kaki*	Taishu	male	bud	15-Oct	SR50
smZenF26Apr	*D. kaki*	Zenjimaru	female	whole flower	26-Apr	SR50
smZenF4Apr	*D. kaki*	Zenjimaru	female	whole flower	4-Apr	SR50
smZenF9Jun	*D. kaki*	Zenjimaru	female	bud	9-Jun	SR50
smZenM26Apr	*D. kaki*	Zenjimaru	male	whole flower	26-Apr	SR50
smZenM4Apr	*D. kaki*	Zenjimaru	male	whole flower	4-Apr	SR50
smZenM4AprZeb	*D. kaki*	Zenjimaru	male	zebularine treated flower	4-Apr	SR50
smZenM9Jun	*D. kaki*	Zenjimaru	male	bud	9-Jun	SR50

Supplemental Table 7: Bisulfite amplicon sequencing sample information.

Library	Species	Cultivar/Accession	Gender	Organ	Time	Amplicons[a]
MiSeq						
PerMet2-1	*D. kaki*	Taishu	female	sepal	2-May	MeGI promoter
PerMet2-2	*D. kaki*	Taishu	female	petal	2-May	MeGI promoter
PerMet2-3	*D. kaki*	Taishu	male	stem	2-May	MeGI promoter
PerMet2-4	*D. kaki*	Taishu	female	stem	2-May	MeGI promoter
PerMet2-5	*D. kaki*	Taishu	male	leaf	2-May	MeGI promoter
PerMet2-6	*D. kaki*	Taishu	female	leaf	2-May	MeGI promoter
PerMet2-7	*D. lotus*	Kunsenshi male	male	stem	2-May	MeGI promoter
PerMet2-8	*D. lotus*	Kunsenshi female	female	stem	2-May	MeGI promoter
PerMet2-9	*D. lotus*	Kunsenshi male	male	bud	2-May	MeGI promoter
PerMet2-10	*D. lotus*	Kunsenshi female	female	bud	2-May	MeGI promoter
TA_Meth-1	*D. lotus*	KK-L1	male	whole flower	23-Apr	MeGI promoter
TA_Meth-2	*D. lotus*	KK-L8	male	whole flower	23-Apr	MeGI promoter
TA_Meth-3	*D. lotus*	KK-L20	female	whole flower	23-Apr	MeGI promoter
TA_Meth-4	*D. lotus*	KK-L18	female	whole flower	23-Apr	MeGI promoter
TA_Meth-5	*D. kaki*	Taishu	male[b]	whole flower	23-Apr	MeGI promoter
TA_Meth-6	*D. kaki*	Taishu	female[b]	whole flower	23-Apr	MeGI promoter
TA_Meth-7	*D. kaki*	Taishu	male[c]	whole flower	23-Apr	MeGI promoter
TA_Meth-8	*D. kaki*	Taishu	female[c]	whole flower	23-Apr	MeGI promoter
TA_Meth-25	*D. lotus*	KK-L1	male	leaf	23-Apr	MeGI promoter
TA_Meth-26	*D. lotus*	KK-L1	male	stem	23-Apr	MeGI promoter
TA_Meth-27	*D. kaki*	Taishu	male	petal	2-May	MeGI promoter
TA_Meth-28	*D. kaki*	Taishu	male	residual ovary	2-May	MeGI promoter
TA_Meth-29	*D. kaki*	Taishu	male	stamen	2-May	MeGI promoter
TA_Meth-30	*D. kaki*	Taishu	male	residual pistil	2-May	MeGI promoter
TA_Meth-31	*D. kaki*	Taishu	female	residual stamen	2-May	MeGI promoter
TA_Meth-32	*D. kaki*	Taishu	female	pistil	2-May	MeGI promoter
HiSeq						
bisTaiFApr23	*D. kaki*	Taishu	female	whole flower	23-Apr	MeGI and OGI promoter
bisTaiFAug13	*D. kaki*	Taishu	female	bud	13-Aug	MeGI and OGI promoter
bisTaiFJan	*D. kaki*	Taishu	female	bud	15-Jan	MeGI and OGI promoter
bisTaiFJun13	*D. kaki*	Taishu	female	bud	13-Jun	MeGI and OGI promoter
bisTaiMApr23	*D. kaki*	Taishu	male	whole flower	23-Apr	MeGI and OGI promoter
bisTaiMAug13	*D. kaki*	Taishu	male	bud	13-Aug	MeGI and OGI promoter
bisTaiM_Jan	*D. kaki*	Taishu	male	bud	15-Jan	MeGI and OGI promoter
bisTaiMJun13	*D. kaki*	Taishu	male	bud	13-Jun	MeGI and OGI promoter
bisTaiMleaf-plus	*D. kaki*	Taishu	male	leaf	2-May	OGI promoter
bisTaiMstem	*D. kaki*	Taishu	male	stem	2-May	MeGI and OGI promoter
MetTaiFApr4	*D. kaki*	Taishu	female	whole flower	4-Apr	MeGI promoter
MetTaiF_13Jun	*D. kaki*	Taishu	female	bud	13-Jun	MeGI promoter
MetTaiF_Jan	*D. kaki*	Taishu	female	bud	15-Jan	MeGI promoter
MetTaiMApr4	*D. kaki*	Taishu	male	whole flower	4-Apr	MeGI promoter
MetTaiM_12Aug	*D. kaki*	Taishu	male	bud	12-Aug	MeGI promoter
MetTaiM_13Jun	*D. kaki*	Taishu	male	bud	13-Jun	MeGI promoter
MetTaiM_Jan	*D. kaki*	Taishu	male	bud	15-Jan	MeGI promoter
MetZenM_4Apr	*D. kaki*	Zenjimaru	male	whole flower	4-Apr	MeGI promoter
FudeCont1	*D. kaki*	Fudegaki	male	whole flower	2-May	MeGI promoter and matK
FudeCont2	*D. kaki*	Fudegaki	male	whole flower	2-May	MeGI promoter and matK
FudeZeb1	*D. kaki*	Fudegaki	male	whole flower	2-May	MeGI promoter and matK

FudeZeb2	*D. kaki*	Fudegaki	male	whole flower	2-May	MeGI promoter and matK
KFApr23_allMeGI_matk	*D. lotus*	Kunsenshi female	female	whole flower	23-Apr	MeGI promoter and matK
KMApr23_allMeGI_matk	*D. lotus*	Kunsenshi male	male	whole flower	23-Apr	MeGI promoter and matK
KM_petal	*D. lotus*	Kunsenshi male	male	petal	2-May	MeGI promoter and matK
KM_pistil	*D. lotus*	Kunsenshi male	male	pistil	2-May	MeGI promoter and matK
KM_sepal	*D. lotus*	Kunsenshi male	male	sepal	2-May	MeGI promoter and matK
KM_stamen	*D. lotus*	Kunsenshi male	male	stamen	2-May	MeGI promoter and matK
MatK_TaiF_Pet	*D. kaki*	Taishu	female	petal	2-May	matK
MatK_TaiF_Pis	*D. kaki*	Taishu	female	pistil	2-May	matK
MatK_TaiF_Sta	*D. kaki*	Taishu	female	stamen	2-May	matK
MatK_TaiM_Pet	*D. kaki*	Taishu	male	petal	2-May	matK
MatK_TaiM_Pis	*D. kaki*	Taishu	male	pistil	2-May	matK
MatK_TaiM_Sta	*D. kaki*	Taishu	male	stamen	2-May	matK
TaiCont	*D. kaki*	Taishu	male	whole flower	2-May	MeGI promoter and matK
TaiFApr23_allMeGI_matk	*D. kaki*	Taishu	female	whole flower	14-Apr	MeGI full-length and matK
TaiFApr_MeGIpro_matk	*D. kaki*	Taishu	female	whole flower	4-Apr	MeGI promoter and matK
TaiFAug_MeGIpro_matk	*D. kaki*	Taishu	female	whole flower	13-Aug	MeGI promoter and matK
TaiFJan_MeGIpro_matk	*D. kaki*	Taishu	female	whole flower	15-Jan	MeGI promoter and matK
TaiFJun_MeGIpro_matk	*D. kaki*	Taishu	female	whole flower	13-Jun	MeGI promoter and matK
TaiHerm	*D. kaki*	Taishu	male	whole flower	2-May	MeGI promoter and matK
TaiMApr23_allMeGI_matk	*D. kaki*	Taishu	male	whole flower	23-Apr	MeGI full-length and matK
TaiMApr_MeGIpro_matk	*D. kaki*	Taishu	male	whole flower	4-Apr	MeGI promoter and matK
TaiMAug_MeGIpro_matk	*D. kaki*	Taishu	male	whole flower	13-Aug	MeGI promoter and matK
TaiMJan_MeGIpro_matk	*D. kaki*	Taishu	male	whole flower	15-Jan	MeGI promoter and matK
TaiMJun_MeGIpro_matk	*D. kaki*	Taishu	male	whole flower	13-Jun	MeGI promoter and matK
TaiZeb1	*D. kaki*	Taishu	male	whole flower	2-May	MeGI promoter and matK
TaiZeb2	*D. kaki*	Taishu	male	whole flower	2-May	MeGI promoter and matK
ZenCont1	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK
ZenCont2	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK
ZenCont2015	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK
ZenZeb1	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK
ZenZeb2	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK
ZenZeb2015	*D. kaki*	Zenjimaru	male	whole flower	2-May	MeGI promoter and matK

[a] Sense and antisense strands were mixed.
[b] Derived from female parent branches.
[c] Derived from male parent branches.

Supplemental Table 8: Primer sequences

primer	sequences (5'-3')	targeted genes or regions	note
PCR for amplification of *OGI* and its 5' promoter genomic region in *Diospyros* species			
OGI-candF1	CACAGTAGTCATATATTTTTAGC	*OGI,* almost all genomic region	OGI-prom2-F-TOPO and OGI-spR for pOGI-*OGI* in Fig. S3c
OGI-spR	CTGGCACACAAAATATTTTCAACCCT		
OGI-prom2-F-TOPO	CACCAAGTATTGATTTTTATTGTACCATTGCTTAT	*OGI,* 5' promoter region	
OGI-prom2-R	AATAGTTACATTACTGGCATGGAATGGGTTAA		
OGI-SINE-Ins-F	AACTGCCCAGGGGTACAACTAAG	Kali SINE insertion	specific to *D. kaki* Kali-in in Fig. S3c
OGI-SINE-Ins-R	TATTATTATGCTCCAACACTCGCAC		
Bisulfite PCR analysis			
***MeGI* 5' promoter and genic region**			
MeGI-SenseProm-bis-F1	GTGTTTTGGTTAAATTAAGTTAATTTAATG	*MeGI* 5' promoter sense direction	
MeGI-SenseProm-bis-R1	CTTTAATCAAAAAATTAAAATTAACTATCATTTT		
MeGI-pro-up-bisSS-F1	TTGTAATTTTGATTTGTATTTTTTATAAG		
MeGI-pro-up-bisSS-R1	TTAAAAACCCCATATCAAACCTT		
MeGI-pro-up-bisSS-F2	TGATAGTGGTATTTTTGGTAATTAGG		
MeGI-pro-up-bisSS-R2	AATATCAAAAAATAATCCTCATAAATAAA		
bisMeGI-prom-SS-re-1F	AGTGATTTTAATAATATTATTGATTAGTTAGTTAGGGTT		
bisMeGI-prom-SS-re-1R	CTCCAAAAACTTATTATAATCTTATTATTTACAA		
bisMeGI-prom-SS-re-2F	GATATTTGTAAATAATAATGATTATAATAAGTTT		
bisMeGI-prom-SS-re-2R	CAAACAAATACATAATAAATAATTAATAATTAATTACCTT		
bisMeGI-5UTR-SS-re-1F	TTATTATATTATAATTTTTTTGTAGTTTTTAAGA	*MeGI* ORF sense direction	
bisMeGI-ORF-SS-re-1R	CTTCCCCCCTCTCCTTTATTCTTCTTCC		
bisMeGI-ORF-SS-re-2F	ATTATATATTTTTATAATAATAATAATTGAATAATTAATG		
bisMeGI-ORF-SS-re-2R	AATACTCCTCTTCTAACTTCTTACTCTTCCATC		
bisMeGI-ORF-SS-re-3F	GTTTGGTTTTAGAATAGAAGGGTTTGATGGAAGAG		
bisMeGI-ORF-SS-re-3R	CACACAAAAAATATTTTCCAATCCTTACATCCCAAACTC		
bisMeGI-ORF-SS-re-4F	TATTGGAGGTTATTAGATGTGATGGGGTTT		
bisMeGI-ORF-SS-re-4R	TCTTAATCACCCTCTAAAAAACAATAAAT		
MeGI-ASProm-bis-F1-N2	ATGAGAGATGAGAGTTATTTGATGATT	*MeGI* 5' promoter antisense direction	
MeGI-ASProm-bis-R1-N2	CTATAACAAATTTCTAAAACTAATTCTTTATCTCC		
MeGI-pro-up-bisAS-F1	TGTTTTTGGAGATAAAGAATTAGTTTTAG		
MeGI-pro-up-bisAS-R1	CCAATATAAAACTTCCAACCTTTATAAATC		
MeGI-pro-up-bisAS-F2	GTAATTGTAGTTGGAAGTTATTAAGGTTA		
MeGI-pro-up-bisAS-R2	TTATAATTTCAACCTACACTCTCTACAAATC		
bisMeGI-prom-AS-re-1F	CCAAATCACAAACCATACTTCAAAACATC		
bisMeGI-prom-AS-re-1R	GGGGGTGTTGTTGGGGAGGATGGAG		
bisMeGI-prom-AS-re-2F	ACTCCTCCCTCCATCCTCCCCAACAA		
bisMeGI-prom-AS-re-2R	ATTTTTGAGGGAGAGATAGAAATGTG		
bisMeGI-5UTR-AS-re-1F	CACATTTCTATCTCTCCCTCAAAAAT	*MeGI* ORF antisense direction	
bisMeGI-ORF-AS-re-1R	GTGTATTAATTATTTGATTATTATTATTATAAGAATGT		
bisMeGI-ORF-AS-re-2F	CACCATTATACATTCTTATAATAATAATAATC		
bisMeGI-ORF--AS-re-2R	GAATATTGTGTTATTGTGTAAGAGAGAGAGTGA		
bisMeGI-ORF-AS-re-3F	CACTCTCTCTCTTACACAATAACACAATATTCA		
bisMeGI-ORF-AS-re-3R	ATTAATGTAATTGTTTTTTGGTATATAAAAAATATTTT		
bisMeGI-ORF-AS-re-4F	ATCAACAACCCAATAAATTCTTCACTTTCAAT		
bisMeGI-ORF-AS-re-4R	TAAAAAATAATAAATGTTAGAGATATTTAAT		

***OGI* 5' promoter**			
OGI-prom-bis-SS-PreIns-F1	AGTTTAGTTATGGAAGAGATTTGTTATGTTG	*OGI* 5' promoter sense direction	
OGI-prom-bis-SS-PosIns-R1	CTTCTTATCATTAATTATTAATTCCCAA		
OGI-prom-bis-SS-Ins-R1	TTTTTTTTTTATTATTATACTCCAACACTC		
OGI-SSPro-bis-ORFR1	AATTACATTACTAACATAAAATAAATTAACC		
OGI-SSPro-bis-endInsF1	AGGAGTGGATTTATTAAGGGTGTG		
OGI-prom-bis-AS-PreIns-F1	GGTTGTTGATTTTTAAAAGTTATGTG	*OGI* 5' promoter antisense direction	
OGI-prom-bis-AS-PosIns-R1	TACTTTTCATATATTAATATAATAAAATTAAACTCC		
OGI-prom-bis-AS-Ins-F1	GTTATGTGATAAATTTTTTTTTTTTTATTATTATG		
OGI-ASPro-bis-ORFF1	TAGAAAATAGTTATATTATTGGTATGGAA		
OGI-ASPro-bis-endInsR1	CTCTCAATTTACTTCTCTCATAATAACTC		
for matK (normalization)			
DkmatK-1F-bis-cont	TGTTGATGAATAAATGGAAATATTA	*matK* ORF sense direction	
DkmatK-1R-bis-cont	TCCTAATACATTACAAAATTTCACTTTA		
qPCR expression test			
MeGI-ov1stInt-F	GACACCACGGAGAAGTAGTGAT	*MeGI* transcript	Designed to bridge the two introns of *MeGI*
MeGI-ov2ndInt-R	GTTCTTTGAGCTTTAGCTCCGTTTC		
MeGI-RT-2ndExon-F1	GTCAGGTGGCCGTTTGGTTTCA		Designed to amplify the 2nd exon
MeGI-RT-2ndExon-R1	TTGGAATACTCCTCTTCTAGCTTCTTGC		
DkActin-F	CATGGAGAAAATCTGGCATCATAC	*Actin* (AB473616)	High expression in all organs tested
DkActin-R	GAAGCACTGGGTGCTCTTCTG		