Supporting Information

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SI Materials and Methods

Phylogenetic Inference of Gene Family Evolution. Predicted amino acid sequences were aligned with broadly sampled sequences from different MIKC-MADS box lineages and from different plant species (Table S1). The best-scoring maximum-likelihood tree was obtained using RAxML (1) with an LG amino acid substitution model (2), and 1,000 bootstrap replicates were estimated using the rapid bootstrap algorithm (3). The bootstrap percentages were summarized from all 1,000 bootstrap trees, and the bipartition tree was obtained by mapping these bootstrap percentages to the best-scoring maximum-likelihood tree.

Microscopy and Image Analysis. For scanning electron microscopy, fixed young floral buds and selected organs were dissected and postfixed in 2% OsO_4 for 2 h, then critically point dried using Samdri-PVT-3D (Tousimis Research). The dried specimens were mounted and sputter-coated with Pd/Pt at 20 mA or Pt/Au at 40 mA for 180 s. The stubs were examined with EVO-SEM at 10.00 keV and image brightness and contrast were uniformly adjusted in Photoshop.

Histology. Specimens were embedded in Kulzer's Technovit (2-hydroethyl methacrylate) for serial microtome sections. A stepwise infiltration was performed with 50:50, 25:75, and 0:100 ratios of 100% ethanol (vol/vol) to Technovit solution. Embedded material was sectioned using a Microm HM 355 Rotary microtome with a conventional knife D. The 7-μm-thick sections were stained with Ruthenium red and Toluidine blue and mounted in Histomount (Invitrogen). Permanent slides of the microtome sections are deposited at the Harvard University Herbaria (A).

1. Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22(21):2688–2690.
2. Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. *Mol Biol Evol* 25(7):1307–1320.
3. Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* 57(5):758–771.

Fig. S1. Illustrations of *Rafflesia cantleyi* (*A* and *B*), *Sapria himalayana* (*C–F*), and *Rhizanthes lowii* (*G–K*). (*A*) Longitudinal section of an open male *Rafflesia* flower, showing the floral chamber enclosing the central disk (cd); ramenta (ra) line the inner surface of the chamber. (*B*) Central disk (cd) of *Rafflesia* with ring (blue); perianth tube (yellow) and bracts (red) have been removed. (*C*) Longitudinal section of an open male *Sapria* flower, showing the floral chamber enclosing the central disk (cd); ramenta (ra) are found on top of the diaphragm. (*D*) Longitudinal section of an advanced female bud of *Sapria*, showing the position of the ovary (ov) and the longitudinal grooves and ridges. (*E*) Longitudinal section of an advanced male bud of *Sapria*, showing the partitioning of the floral chamber into two compartments by the diaphragm (dia). (*F*) Detail of the ramenta on the upper surface of the diaphragm in *Sapria*. (*G*) Longitudinal section of an open male *Rhizanthes* flower, showing the exposed central disk. (*H*) Detail of the pads on the perianth lobes in *Rhizanthes*. (*I*) Longitudinal section of an advanced female bud of *Rhizanthes*, showing the position of the ovary; tails are removed. (*J*) Longitudinal section of an advanced male bud of *Rhizanthes* with tails (ta) shown (ovarial locules without functional ovules present). (*K*) Detail of the pads on the perianth lobes of an open *Rhizanthes* flower relative to the perianth tube, which is the region below the band of long hairs.

Fig. S1 (PDF)

Fig. S2. Gene trees of MADS-box sequences from Rafflesiaceae and outgroup families. Sequences generated for this study are in red. Maximum likelihood support above 50% is indicated at the nodes. (*A*) MADS box gene tree based on the MADS intervening keratin-like (MIK) domains, with gene lineages colored as follows: A- (yellow), B- (blue), C-/D- (red), and E-class (green). (*B*) *GLOBOSA*-like genes. (*C*) *DEFICIENS*-like genes. *Inset* in the upper left corner depicts the consensus on the duplication history of the locus in angiosperms [after Jaramillo and Kramer (1)]. (*D*) *AGAMOUS*-like genes. *Inset* in the upper left corner depicts the consensus on the duplication history of the locus in angiosperms [after Jaramillo and Kramer (1)]. (*E*) *SQUAMOSA*-like genes. *Inset* in the upper left corner depicts the consensus on the duplication history of the locus in angiosperms [after Litt and Irish (2)]. (*F*) *SEPALLATA*-like genes.

Fig. S2 (PDF)

1. Jaramillo MA, Kramer EM (2007) The role of developmental genetics in understanding homology and morphological evolution in plants. *Int J Plant Sci* 168(1):61–72.
2. Litt A, Irish VF (2003) Duplication and divergence in the APETALA1/FRUITFULL gene lineage: Implications for the evolution of floral development programs. *Genetics* 165(2):821–833.

Fig. S3. PaleoAP3 and euAP3 C-terminal motifs of *DEF*-like genes from Rafflesiaceae and outgroups. Note the extensive stretch of amino acids preceding the paleoAP3 motif in the *TM6* homologs of Rafflesiaceae (gray shading).

Fig. S3 (PDF)

Fig. S4. Sampling scheme for *Rafflesia* (*A*), *Sapria* (*B*) and *Rhizanthes* (*C*). Labels correspond to those in Fig. 2: Br, bracts; Bs, base of the flower; Dia, diaphragm; Dsk, disk; Lb, perianth lobes; ; Ov, ovary; St, stamens; Tb, perianth tube. Taxon-specific regions for *Rafflesia*: Flr, floor of the floral chamber. Taxon-specific regions for *Sapria*: In, inner perianth lobes; Out, outer perianth lobes. Taxon-specific regions for *Rhizanthes*: Tl, tails, PrD, E and F, levels indicated in Fig. S6*C*. Note that because of their alternate positions in *Sapria*, an outer perianth lobe is depicted on the left side and an inner perianth lobe is depicted on the right side of the longitudinal section of the flower.

Fig. S4 (PDF)

Fig. S5. MADS-box gene expression in Rafflesiaceae and *Clutia* (Peraceae). Actin is used as a concentration control. Column labeling corresponds to the floral regions in Fig. S4. (*A*) *Rafflesia* MADS-box genes. *RfSTK* is expressed in two splice variants, with or without exon 6, which generates two bands in RT-PCR. (*B*) *Sapria* MADS-box genes. (*C*) *Rhizanthes TM6*. (*D*) Expression of MADS-box genes in *Clutia* flowers: Car, carpels; Pe, petals; Se, sepals. Primers are not designed to discriminate between the duplicate copies/alleles of *CluPI* (yellow), *CluTM6* and *CluAG* (blue). Gray shading indicates the uncertainty in the gene expression profile of *Clutia* nectaries.

Fig. S5 (PDF)

Fig. S6. Light microscopy images of sections of buds of *Rafflesia* (*A*) and *Rhizanthes* (*B*–*G*). (*A*) Longitudinal section of *Rafflesia* bud, showing the appearance of stamens (asterisk) and the ring structure outside of the stamens (arrowheads). (*B*) Longitudinal section of a *Rhizanthes* bud, showing the growth of the ring (arrowhead); fusion (not certain to what extent congenital or postgenital) between the ring and the perianth lobe is depicted by arrows. A stamen is marked with an asterisk. (*C*) Longitudinal section of an advanced *Rhizanthes* bud; dotted lines along the right side of the section correspond to the transverse sections shown in *D*–*G*. Adnation of the ring derivative to the perianth lobe, at levels D–F, is depicted by arrows; level G is at the perianth tube. A stamen is marked with an asterisk. (*D*) Transverse section at the level where the perianth lobes are completely free and arc inwards toward the central column to form the tails. Arrows indicate the adnation of the perianth lobes with the distal end of the ring derivative. (*E*) Transverse section at the level of the pads. Fusion between the pads and the perianth is discernible (arrows); a complete dissection of this compound structure is indicated with a dashed line. (*F*) Transverse section at the level where the pads fuse laterally to form an uninterrupted band (black dots) on the inner side of the perianth lobes, which remain free (dashed line). Fusion between the pads and the perianth lobes is still noticeable (arrows). (*G*) Transverse section through the perianth tube, which is more homogeneous and no dissection or fusion is observed. (Scale bars, 500 μm.)

Fig. S6 (PDF)

Fig. S7. Evolutionary models for the possible origin of floral chambers in Rafflesiaceae. (*A*) Independent origin of floral chambers in *Sapria* and *Rafflesia*, in which the common ancestor lacked chamber closure. (*B*) Origin of a *Rafflesia*-like floral chamber from a *Sapria*-like floral chambered ancestor via developmental system drift with the loss of the floral chamber in *Rhizanthes*. (*C*) A model where the common ancestor of Rafflesiaceae possessed a floral chamber, which was lost in the common ancestor of *Rafflesia* + *Rhizanthes*. Subsequently, a floral chamber with different organization re-evolved in *Rafflesia*.

Fig. S7 (PDF)

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)