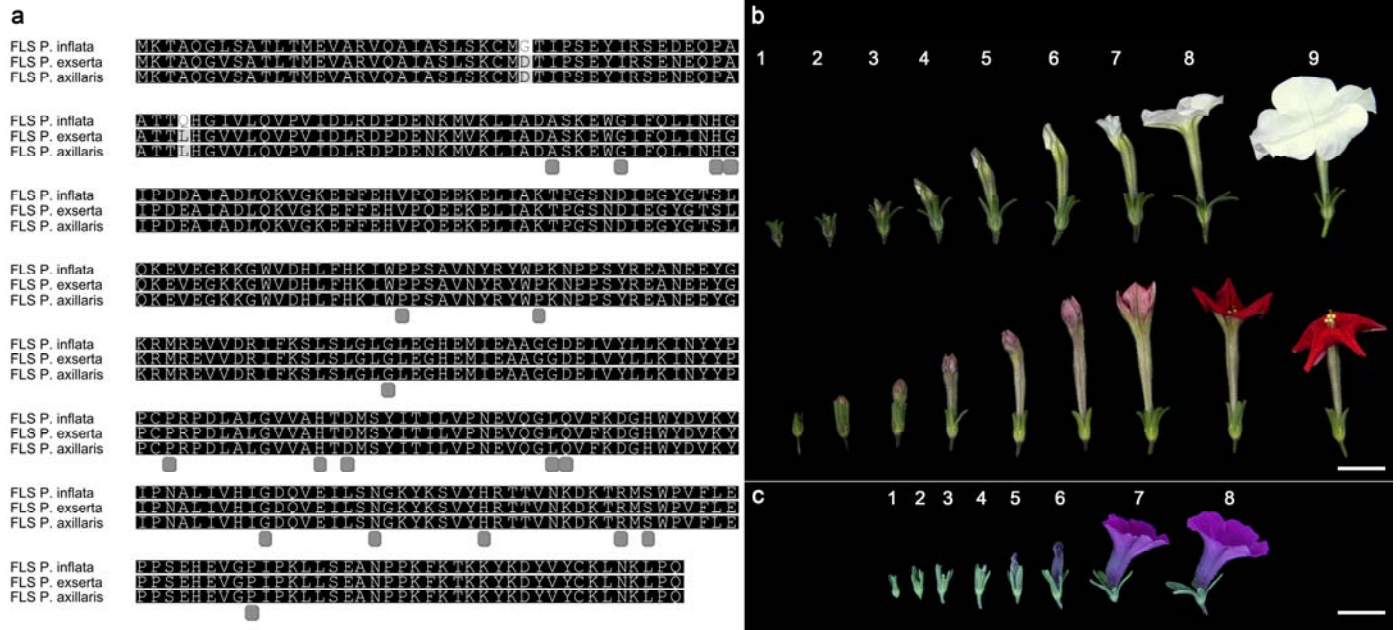


Supplementary Figure 1

**UV-absorbance differs significantly between *P. axillaris* Rio Arapey (Arapey) and *P. axillaris* S7 (S7) but floral traits important for pollinator attraction do not differ substantially.**

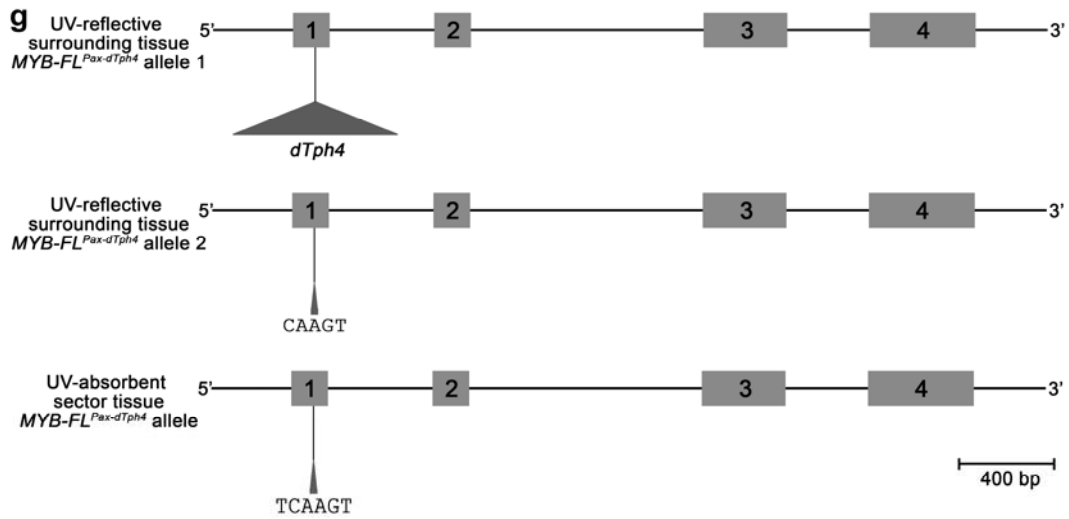
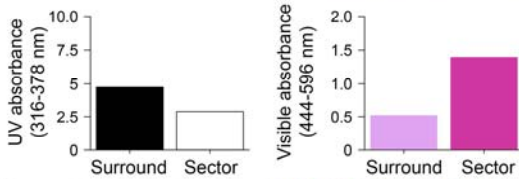
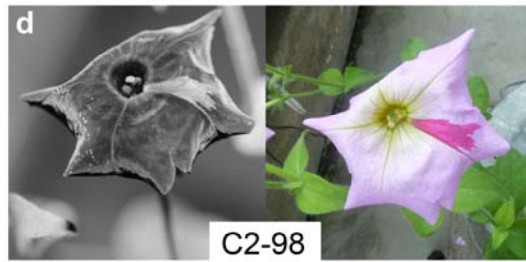
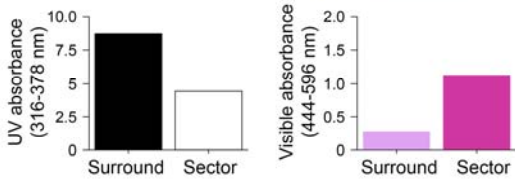
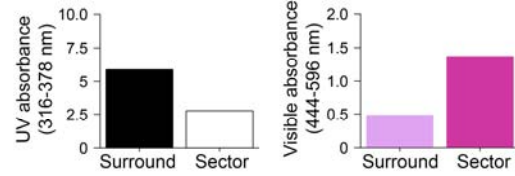
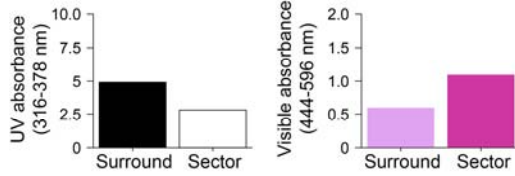
(a) UV-absorbance of the corolla limb. (b) Visible absorbance of the corolla limb. (c) Nectar volume. Note that the difference between Arapey and S7 does not affect the time spent feeding (Fig. 1e). (d) Quantity of scent (methylbenzoate) emission. Scent had high variability within S7 but this did not affect the overall preference of *M. sexta* for Arapey flowers (Fig. 1c,d). (e) Surface area of the corolla limb. Bars show mean±SD; for Rio Arapey samples, (a,b,e)  $n = 5$ , (c)  $n = 4$ , (d)  $n = 6$ ; for S7 samples, (a-d)  $n = 4$ , (e)  $n = 5$ . Statistical tests were carried out using a student's t test ( $\alpha = 0.05$ ).



Supplementary Figure 2

**Protein sequence alignment of flavonol synthase in *P. inflata*, *P. exserta* and *P. axillaris*, and developmental series of floral buds used for gene expression analyses.**

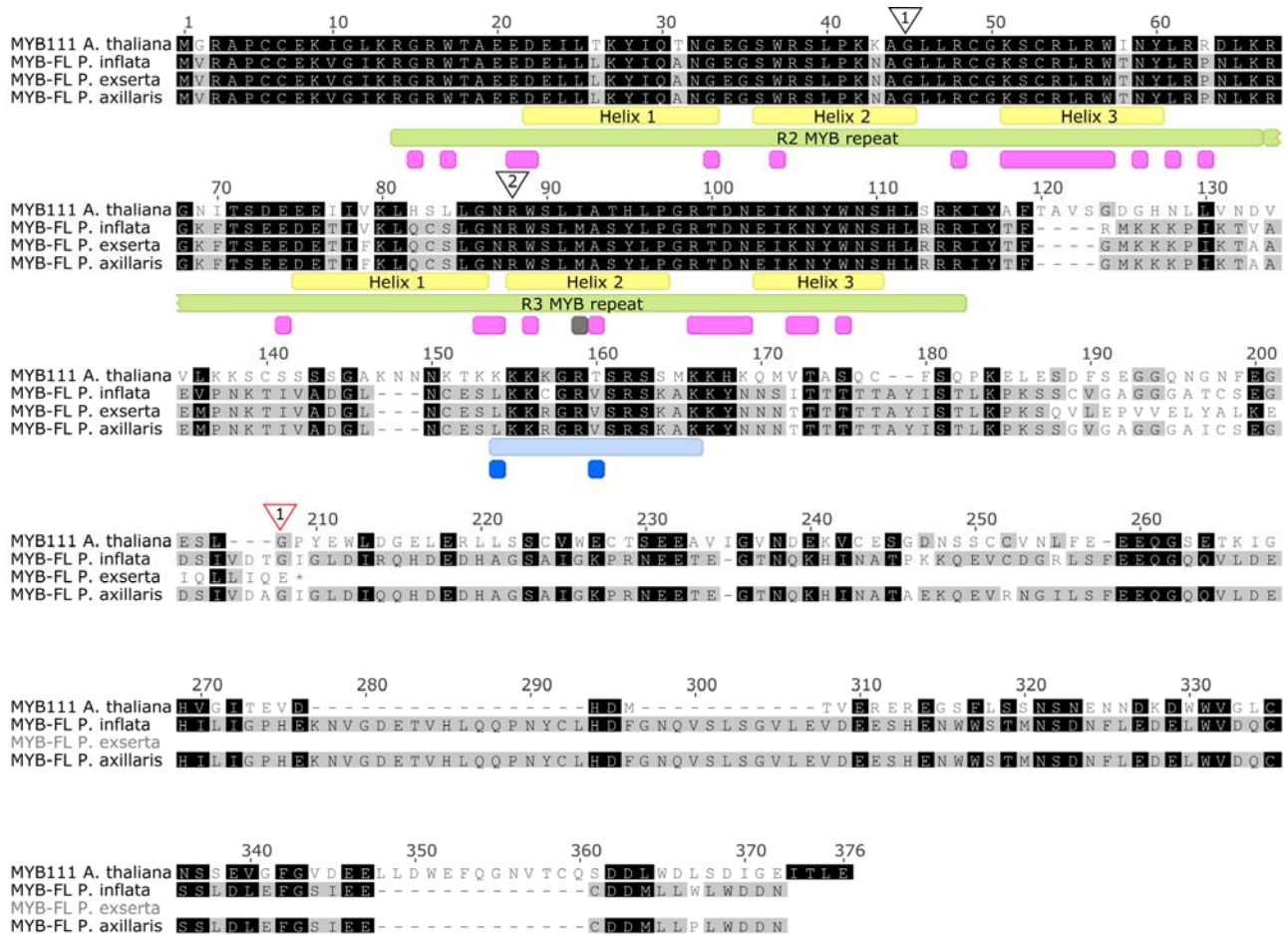
(a) Flavonol synthase (FLS) is a member of the 2-oxoglutarate iron-dependent oxygenases (2-ODD)<sup>43</sup>. The predicted protein sequence of FLS shows no differences between *P. axillaris* and *P. exserta*, and only two residue changes between *P. inflata* and *P. axillaris/P. exserta*. Neither residue corresponds to conserved and/or functionally important residues, making differences in enzymatic activity unlikely. Dark grey boxes indicate residues that are conserved between 2-ODD proteins from different plant species<sup>87</sup>. His34, Asp36 and His290 are required for iron binding, and Arg300 and Ser302 bind the 2-oxoglutarate substrate<sup>88</sup>. (b,c) Developmental stages of floral buds of *P. axillaris* (b, top), *P. exserta* (b, bottom) and *P. inflata* (c). Numbers define the stages of development, as used for quantitative RT-PCR and RNA-seq experiments. For *P. axillaris* and *P. exserta*, stages are separated by approximately one day each. For *P. inflata*, stages 2-4 are separated by one to two days each, and stages 4-8 are separated by approximately one day each. Scale bars represent 2 cm.



### Supplementary Figure 3

#### Appearance of various somatic and germline mutants in UV and visible light including a revertant sector and details of associated *MYB-FL* alleles.

(a-d) Four examples of sectors obtained from *P. axillaris* x W138 F<sub>1</sub> (a,c) and IL2-1<sup>Pax</sup> x W138 F<sub>1</sub> (b,d) individuals. Each example is shown in UV light (left) and visible light (right), with UV and visible absorbance measurements below. (e) A flower from the germline mutant, A1-95 (genotype: *MYB-FL*<sup>Pax-dTph4</sup>/*MYB-FL*<sup>W138</sup>), shown in UV (left) and visible (right) light. (f) The germline mutant, A1-95, was crossed with *P. exserta* to test for genetic complementation (Figure 4e) and one of the progeny from this cross that is heterozygous for *MYB-FL*<sup>Pax-dTph4</sup> and *MYB-FL*<sup>Pex</sup> is shown in UV (left) and visible light (right). This individual has a primarily UV-reflective corolla limb but a UV-absorbent sector is present caused by excision of the *dTph4* transposon from the *MYB-FL*<sup>Pax-dTph4</sup> allele. (g) DNA was extracted from the sector and the surrounding corolla tissue of the flower shown in (f), and the alleles of *MYB-FL* that were derived from *P. axillaris* were sequenced from each of these different tissues and are represented in the schematic. The UV-reflective surrounding tissue showed two alleles: allele 1 contained the *dTph4* transposon and allele 2 contained a 5 bp footprint left by excision of this transposon (which ultimately causes a frameshift resulting in 21 non-homologous amino acid residues before a stop codon). In the UV-absorbent sector tissue, an allele with a 6 bp footprint due to *dTph4* excision was found. This results in the addition of two amino acid residues (Phe and Lys) but the protein sequence remains otherwise intact allowing the *MYB-FL*<sup>Pax-dTph4</sup> allele to return to a functional state after excision of the *dTph4* transposon.



Supplementary Figure 4

**Protein sequence alignment of MYB111 from *Arabidopsis* and MYB-FL from *P. inflata*, *P. exserta* and *P. axillaris*.**

The N terminus of R2R3-MYBs is highly conserved, with two repeats (R2 and R3; green) which each contain three  $\alpha$ -helices (yellow) and are involved in DNA-binding, whereas the C terminus is typically less conserved<sup>89</sup>. The motif that defines subgroup 7 of the R2R3-MYBs in *Arabidopsis* (AtMYB11, AtMYB12 and AtMYB111; [K/R][R/x][R/K]xGRT[S/x][R/G]xx[M/x]K) is present in *Petunia* sequences (light blue) with two mismatches (dark blue)<sup>90,91</sup>. Residues that were conserved in the N-terminus in over 90% of the R2R3-MYBs from *Arabidopsis* that were analysed are shown in purple<sup>90</sup>. Only one was not conserved in the *Petunia* sequences (grey). The positioning of the first two introns (black triangles) is the most common intron pattern in R2R3-MYBs<sup>92</sup> and is conserved in the *Arabidopsis* MYB111 and *Petunia* MYB-FL sequences. The presence of a third intron (red triangle) is present only in MYB-FL but conserved in all three alleles. The numbers inside the triangles indicate the phase of the introns. Alignment was made using Geneious (Biomatters) with manual modifications.