Characterizing Flower Organ Identity Genes in a Homeotic Mutant

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Abstract

The ABC model of flower development explains the patterning of organs along the flower axis. Involving mostly MADS box genes that cause homeotic mutations, this model has been the botanical counterpart to the patterning of the animal body plan by HOX genes. The flowering plant species *Thalictrum thalictroides*, in the buttercup family Ranunculaceae, belongs to an interesting lineage that is sister to all other eudicots, including the model system *Arabidopsis thaliana. T. thalictroides* flowers consist of three types of organs; petaloid sepals, stamens and carpels. I will be using a forward genetics approach to study a horticultural mutant of *T. thalictroides*, 'Betty Blake.' Its phenotype consists of multiple whorls of non-petaloid (green) sepals and multiple whorls of carpels, resembling B-class gene *Arabidopsis* mutants. We hypothesize that the reason for the extra carpels and extra, non-petaloid sepals in 'Betty Blake' is either a mutation in one of the B-class genes or a change in the regulation region of expression of the C-class gene *AG1*. The molecular dissection of this mutant will contribute to understanding the degree of conservation of stamen and carpel identity genes across divergent flowering plants, and to testing whether there is sub-functionalization of gene function for petaloidy of sepals.

Introduction

- The classic 'ABCE' model of flower organ identity links the establishment of the four floral organs: sepals, petals, stamens and carpels, to the interaction of the products of these four gene classes. Mutations in these genes are *homeotic*, replacing one type of organ by another. *Arabidopsis* B-class gene mutants exhibit homeotic conversions of petals to sepals and stamens to carpels, while C-class mutants have extra petals and sepals at the expense of reproductive organs.
- The Floral Quartet Model explains how the development of each floral organ requires specific protein tetramers of the 'ABCE' transcription factors.

'ABCE' Model

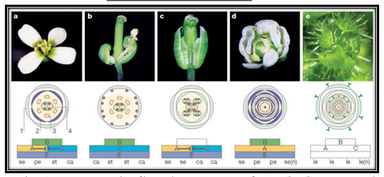


Fig 1. Homeotic floral mutants of *Arabidopsis* and the corresponding 'ABCE' model showing the missing expression for each of different classes.

Floral Quartet Model

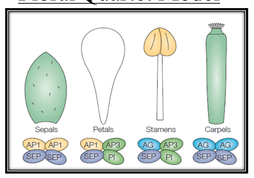
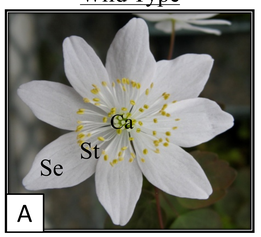


Fig 2. 'ABCE' proteins form tetramers that bind DNA in order to function.

- *T. thalictroides*, commonly known as Rue Anemone, has three organ types: sepals, stamens and carpels. Sepals exhibit a petaloid morphology and are either white or pink.
- 'Betty Blake' is a male-sterile mutant of *T. thalictroides* with only two floral organs: sepals and carpels. In addition, the sepals are green, displaying a more typical leaf-like sepal morphology.

Wild Type



Betty Blake

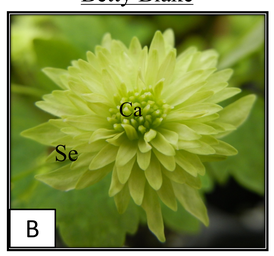


Fig 3 Phenotypes of *T. thalictroides.* (A) Wild Type has petaloid sepals, stamens and carpels. (B) 'Betty Blake' has multiple whorls of smaller, less petaloid sepals, and carpels.

Goal

We set out to dissect the molecular basis of the 'Betty Blake' homeotic flower mutant, using the candidate genes from the ABCE model of flower development.

- **Hypothesis 1**: One or more of the B-class genes, *APETALA3* (*ThtAP3-1, ThtAP3-2a, ThtAP3-2b)* and/or *PISTILLATA* (*ThtPI*), could be dysfunctional in 'Betty Blake.'
- **Hypothesis 2:** 'Betty Blake' could be a weak C-class mutant, with a mutation within the regulatory region of the gene *ThtAGAMOUS1* (*ThtAG1*), that alters its expression domain.

Results

***APETALA3-1* Gene Structure**

Fig 6.
Genomic gene schematic of *AP3-1* in the 'Betty Blake' mutant. The total length is approximately 1.5kb, and contains 7 exons and 6 introns.

***AGAMOUS1* Intron One**

Fig 7.
Genomic gene schematic of wild type *AG1* from the beginning of the first exon to the end of the second exon. Multiple regulatory motifs needed for correct gene function are indicated. The location of the regulatory motifs in *AG1* intron one for 'Betty Blake' are identical.

Key:

- Exons
- Introns
- CCAATCA Box
- aAGAAT Box
- LFY Binding
- CArG Box

B-class genes

ThtAP3-1 was amplified from 'Betty Blake' genomic DNA. The location of the exons was identified by comparison to the orthologous gene from the related species *Aquilegia coerulea.*

C-class gene

Previously amplified sequences of intron one of the C-class gene *ThtAG1* from 'Betty Blake" and wild type were analyzed. The regulatory motifs identified were identical between the two.

Methods and Procedures

DNA was extracted from leaves and flower of both wild type *T. thalictroides* and 'Betty Blake', then amplified by Polymerase Chain Reaction (PCR) using *ThtAP3-1*-specific primers to obtain the genomic locus. Gel Electrophoresis was used on the ~1.5 kb PCR product in order to confirm the length of the desired gene. The PCR product was then ran in a 1% agarose gel and cloned (Invitrogen) TA cloned. Once the gene was sequenced, it was analyzed using Sequencher (v.4.9), McClade and Mesquite.

Discussion

The B-class genes are likely candidates for the phenotype of the 'Betty Blake' flower mutant. Sequencing of this gene, however, did not reveal any major mutations, so we rejected **H1** regarding this particular B class gene. Nevertheless, mutations may still be present in one or more of the three other members of the B class lineage (two additional AP3s and a PI), this will require further investigation.

All of the regulatory motifs identified in the first intron of *ThtAG1* were identical between 'Betty Blake' and wild type. Therefore we reject **H2** based on currently available data. However, we cannot discard the possibility that there may be mutations found within other introns.

Conclusions

In this study we have established the genomic sequence and intron-exon structure of an *AP3* ortholog in a ranunculid, and proceeded to investigate the genetic basis of male-sterile mutants. Or results discard *AP3-1* and the regulatory intron of *AG* as possible culprits and narrow down the candidate gene search to the remaining 3 B-class genes found in *Thalictrum*.

Further Studies

- To further test our hypotheses, we will sequence the remainder of the B-class genes *(AP3-2a,AP3-2b, PI)* and wildtype *AP3-1*, for a more accurate comparison to 'Betty Blake'
- The remainder of *AG1* for 'Betty Blake' should be sequenced, and aligned to wild type as well. The second intron is known to contain regulatory motifs which could vary from wild type.

References

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