Loss of deeply conserved C-class floral homeotic gene function and C- and E-class protein interaction in a double-flowered ranunculid mutant

Kelsey D. Galimba1, Theadora R. Tolkin1, Alessandra M. Sullivan2, Rainer Melzer4, Günter Theißen4, and Verónica S. Di Stilio3,5

1Department of Biology, University of Washington, Seattle, WA 98195; and 2Department of Genetics, Friedrich Schiller University Jena, D-07743 Jena, Germany

AUTHOR SUMMARY

Comparative studies of genetic programs underlying body patterning in plants and animals have been the focus of much research. In plants, this approach has revealed a surprisingly deep functional conservation of flower organ identity among angiosperms (flowering plants). Current understanding of floral patterning comes from research on model plants belonging to recently derived evolutionary lineages, such as Arabidopsis thaliana and Antirrhinum majus, members of a clade known as the “core eudicots.” In these species, the ABCE model predicts how combinatorial expression of four gene classes encoding transcription factors specifies the identity of the different floral organs (i.e., petals, stamens, carpels, and sepals) in the floral meristem (1). [A- and E-class proteins specify sepals; A-, B-, and E-class proteins specify petals; B-, C-, and E-class proteins specify stamens; and C- and E-class proteins specify carpels and terminate floral meristem development (1–2).] The underlying biochemical mechanism for specifying organ identity has been described by the floral quartet model, which predicts that correct transcription of organ-specific genetic programs requires the formation of protein complexes among these four interacting classes of transcription factors (3). Mutations affecting class A, B, C, and E functions are homeotic, resulting in the replacement of one organ type by another. Loss of expression of the Arabidopsis C-class gene AGAMOUS (AG) results in the conversion of reproductive organ fate (stamens and carpels) to sterile petal and sepal fate and floral meristem indeterminacy (4). These showy, nested flowers with excess petals are commonly known as “double flowers.”

The double flower was the first type of flower mutation documented, at least 2,000 y ago (5), and double-flowered cultivars (e.g., roses, peonies, carnations, and camellias) have become popular garden plants because of the attractiveness imparted by the extra petals. Similar natural deviations from normal development have been used to help elucidate the genetic basis of normal flower development. Although a variety of double-flowered cultivars have been described, to our knowledge there is no functional evidence for the underlying molecular mechanism of this familiar phenotype other than in model systems. Thalictrum thalictroides includes cultivars that exhibit homeotic floral phenotypes suggestive of defects in the canonical organ-identity genes of the ABCE model. Among them, we identified T. thalictroides ‘Double White’ as a candidate for loss of floral homeotic C-class function, based on its double-flower phenotype. This cultivar is a sterile homeotic mutant with flowers consisting entirely of multiple white petaloid sepals (Thalictrum lacks petals). It presumably occurred spontaneously in natural populations where it was collected and clonally propagated because of the attractiveness of the double flowers.

Here, we set out to characterize the putative AG ortholog ThtAG1 in T. thalictroides functionally, while testing whether a mutation at this locus is responsible for a double-flowered variety. In a reverse genetics approach, targeted silencing of ThtAG1 caused a double-flowered phenocopy of the cultivar. In the complementary forward genetics approach, we gathered strong evidence for the ‘Double White’ cultivar being affected in the AGAMOUS-like gene ThtAG1.

Down-regulation of ThtAG1 by virus-induced gene silencing, a targeted gene-silencing technique, resulted in homeotic conversion of stamen and carpel identity into sepaloid identity and loss of flower determinacy. Moreover, flowers exhibiting strong silencing of ThtAG1 closely resembled double flowers in the ornamental cultivar (Fig. P1). Molecular analysis of ‘Double White’ revealed the insertion of a transposable element into the C-class gene, resulting in the expression of mutant proteins. Biochemical analysis demonstrated that the mutation abolishes protein–protein interactions with the putative E-class protein partner. Heterodimerization of C- and E-class proteins is predicted by the floral quartet model, yet evidence for the functional importance of this interaction is scarce in lineages other than core eudicots. Our finding, that a C-class mutant with a defective protein hampers the interaction with a SEP protein, corroborates the importance and conservation of this interaction.

Author contributions: V.S.D. designed research; K.D.G., T.R.T., A.M.S., and R.M. performed research; K.D.G., T.R.T., R.M., G.T., and V.S.D. analyzed data; G.T. designed the Y2H experiments; and K.D.G., T.R.T., A.M.S., R.M., G.T., and V.S.D. wrote the paper.

The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

Data deposition: The sequences reported in this paper have been deposited in the GenBank Database (accession nos. JQ002519, JQ002520, and JN887118–JN887121).

1To whom correspondence should be addressed. E-mail: distilio@uw.edu.

See full research article on page E2267 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1203686109.

PNAS August 21, 2012 | vol. 109 | no. 34
13478–13479 | PNAS | August 21, 2012 | vol. 109 | no. 34
www.pnas.org/cgi/doi/10.1073/pnas.1203686109
In summary, our results provide strong evidence for high conservation of C-class gene function and of C- and E-class protein interactions between core eudicot model plants and a representative of the sister lineage to all other eudicots, the ranunculids. Comparable roles of a ranunculid C-class gene in reproductive organ identity and flower meristem determinacy were observed. Furthermore, we identified the genetic and biochemical basis of an ornamental double-flowered cultivar, suggesting that mutations in C-class genes likely underlie other widespread double-flowered varieties.