

Background Information for “Reconstruction the Evolution of Cauliflower and Broccoli”

The domestication of crop plants over 10,000 years provides rich opportunities to explore evolution through artificial selection. The subject also places genetic engineering in a broader context, as many students associate the term only with recombinant DNA. The laboratory experience is built around the findings reported by Smith and King (200) and Purugganan, Boyles and Suddith (2000) on the evolution of domesticated *Brassica oleracea* focused on the *CAULIFLOWER* (*CAL*) gene.

Before delving into specifics, it is important to recognize that there are limitations to putting too much weight on one or two genes in understanding evolution. Quantitative trait loci mapping studies reveal the variability in the number and effect of loci, ranging from a couple to many, that affect plant adaptive traits (Ehrenreich and Purugganan 2006). We intentionally chose the *CAL* example because the molecular evolutionary data is compelling and because considering adaptive traits controlled by many loci adds a layer of complexity that leads to confusion rather than learning progress for beginning college students. We have found that examples involving multiple loci are more effectively integrated into our mid-level genetics, development, and evolution courses. Recognizing the difference in single gene and quantitative trait locus approaches also reflects new conceptualizations in the emerging fields of evolution of development and ecological and evolutionary functional genomics. The laboratory experience, as we have designed it, leaves opportunities for further discussion and exploration should the instructor choose to adapt it to an upper level course. Most pointed is the guided inquiry where we lead students to a final conclusion that both broccoli and cauliflower plants have the same stop mutation in the *CAL* gene. Clearly broccoli and cauliflower do not look the same and the only reasonable conclusion one can draw is that there are other genetic differences that have been selected for during the ongoing domestication of cauliflower and broccoli.

With the above caveats about plant adaptive traits, some background information on the *CAL*, the gene of interest in this lab, may be helpful. The gene was first identified through mutant analysis in the model plant *Arabidopsis*, a close relative of the broccoli and cauliflower (Kempin et al. 1995). *Arabidopsis* plants with two copies of the *cal* mutation have a wild type phenotype. It is only in the double mutant *apetala1* (*ap1*) *cal* that the inflorescences (branching portion of the plant containing the flowers) resemble cauliflower rather than the simple, single flower per node architecture of wild type *Arabidopsis*. [Images of these plants and others can be found in the “Description and Teaching Materials” section of the laboratory website.] Sequence analysis reveals that *CAL* and *AP1* have 82% maximum identity (you can try this using one of the *CAL* accession numbers in a BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST/>)). The most likely explanation is a duplication event. *AP1* is highly conserved among flowering plants, thus *CAL* is most likely the duplicated gene that diverged. Our students are intrigued by redundant genes that arise through gene duplication and begin diverging. The redundancy or partial redundancy explains why only the double mutant has the extreme cauliflower phenotype (*ap1* alone does have a noticeable phenotype, but *cal*

does not). The flexibility that redundant genes provide in evolution is worth a brief discussion with students.

Understanding how the duplication gave rise to two identical and, over evolutionary time, similar genes, is a helpful way to begin exploring what paralogous genes are. This naturally leads to an exploration of orthologs and the question of whether or not *CAL* exists in other plants (e.g. has orthologs) and whether the mutant phenotype in *Arabidopsis* is coincidental or relates to the phenotype of cauliflower plants one can purchase in the supermarket.

Lowman and Purugganan (1999) provided phylogenetic evidence that the *cal* mutation arose within the Brassica family. This work and later research also supports the conclusion that the duplication event producing *CAL* occurred within the Brassicas. Two points may be of interest to students based on this conclusion. First, while it is possible that other plant families could evolve inflorescences that look like cauliflower, the responsible gene, even if it were an *AP1* duplicate would not be orthologous to *CAL*, because the families diverged before the Brassica *CAL* gene appeared on the evolutionary scene. The second point is to be clear about the difference between the *cal* mutation that was studied in *Arabidopsis* where mutations are made for developmental genetic studies and the *cal* mutation that naturally arose in the *Brassica oleracea* and were selected for by early agricultural societies. Both are examples of artificial selection. The *Arabidopsis* data can be used to understand the evolution of broccoli and cauliflower, but the starting information from *Arabidopsis* was not causal in the evolution of the subspecies of *Brassica oleraceae*.

In the student version of the laboratory description, there is a paragraph about the role of *AP1* and the double mutant necessary for the cauliflower phenotype. Lowman and Purugganan's (1999) work also supports the role of the double mutant in cauliflower evolution. This paragraph can be eliminated if the instructor chooses to focus exclusively on the *cal* mutation or can be used as a springboard for discussing content outlined in the preceding paragraph in this background information document.

The student laboratory and the link to *Brassica oleracea* images under "Description and Teaching Materials" offer the opportunity to explore the concept of speciation and what constitutes a species. The morphologically obvious difference among the subspecies and the molecular genetic conclusion the students arrive at regarding one aspect of the morphological differences could be used to reinforce what students have already learned about the species concept and agents of evolutionary change if Hardy-Weinberg equilibrium has been covered. That is the approach we have used in the past few years. In past years we have used the laboratory experience as a jumping off point for classroom work on speciation and found that to be effective in terms of student learning as well.

Our students have been involved in a multi-week research project to determine whether or not gall fly speciation is occurring in our local arboretum before they begin the broccoli and cauliflower lab

(http://serc.carleton.edu/sp/carl_ltc/quantitative_writing/examples/labguide.html). They have already worked through real world data, using Hardy-Weinberg and Chi square analyses, to determine whether or not the gall flies are undergoing genetic differentiation for host species preference. While we are able to integrate the two learning experiences during student reflection and discussion times, the “Reconstructing the Evolution of Cauliflower and Broccoli” module is not dependent on the previous laboratory experience.

Our goal is for students to integrate their understanding of genetics, evolution, and development over multiple levels of organization – DNA sequence level to whole plant morphology in this case. For those who prefer to narrow the focus to molecular genetics and evolution, the module should also be effective.

References

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