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An Inquiry-Infused Introductory Biology Laboratory That Integrates Mendel's Pea Phenotypes with Molecular Mechanisms

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Abstract: We developed a multi-week laboratory in which college-level introductory biology students investigate Mendel's stem length phenotype in peas. Students collect, analyze and interpret convergent evidence from molecular and physiological techniques. In weeks 1 and 2, students treat control and experimental plants with Gibberellic Acid (GA) to determine whether uncharacterized short mutant lines are GA responsive. These data allow students to place the mutation in the GA signal transduction pathway. During weeks 2 and 3, plants are genotyped for Mendel's *le* mutation using a derived cleaved polymorphic sequences (dCAPS) PCR assay. This laboratory allows students to make a direct connection between modern molecular genetics and the easily scored phenotypes Mendel used as the basis of his fundamental discoveries. We administered surveys to assess student gains in accord with four learning goals: understanding the lab, basic science literacy, scientific practices, and working collaboratively. Student confidence increased significantly in the first three, but not in working collaboratively, although students reported greater confidence working in groups than alone.

Key words: Laboratory, Genetics, Inquiry-Based Instruction

INTRODUCTION

The official position of the National Science Teachers Association is that an undergraduate laboratory experience "should not be a rote exercise in which students are merely following directions, as though they were reading a cookbook" (NSTA, 2006). Despite this, many undergraduate introductory biology laboratory exercises (including several currently taught at Swarthmore College) are self-contained 3-hour affairs in which students follow prescribed protocols to reproduce known results. One pragmatic rationale for adherence to this traditional paradigm is the ease with which instructors can guide and evaluate the work of a large group of students. Another is interest in exposing students to a broad range of concepts and methods (Anderson, 2002). Student success in these labs is usually defined as the degree to which results conform to predetermined outcomes. Even when successful, such "cookbook" experiences can be discouraging for students as they lack prospects for personal discovery or authentic contributions to science. Traditional labs can present the nature of science as confirmatory rather than exploratory and relegate students to the role of passive audience members rather than active participants (Munby & Roberts, 1998). Pedagogical methods such as these have been described as disengaging and disempowering (Roth & Lee, 2004).

In response to the traditional paradigm, progressive educators have widely advocated inquiry-based science education since at least the early 20th century (Dewey, 1938). Inquiry is marked by exploration of true unknowns, participation in experimental design, time for reflection and revision,

and a capstone such as a written or oral presentation (NRC, 2000) and is most fully realized in mentored student research experiences (Katkin, 2003). Benefits of these experiences include procedural troubleshooting skills and a better understanding of the role of convergent evidence in establishing claims (Bleicher, 1996; Kardash, 2000; Richie & Rigano, 1996; Ryder, et al., 1999). Such gains in concert with positive collaborative relationships with mentors or other group members have the potential to facilitate formation of scientific identities and to stimulate further participation and deeper membership in the scientific community (Hunter et al., 2006; Seymour, et al., 2004; Templin & Doran, 1999) and membership in related communities such as Western medicine (Kudish, 2009). Although independent student research experiences are an effective mode of inquiry-based learning, such experiences are not widely available to introductory biology students due to their high cost in time and attention of faculty mentors or other laboratory members (Merkel, 2003). As a practical compromise, recent curricular innovations have infused inquiry elements into weekly biology labs resulting in improved student edification and satisfaction (Rissing & Cogan, 2009; Lord & Orkwiszewski, 2006).

We set out to design an inquiry-based laboratory appropriate for a college level introductory biology course that would allow students to work directly with one of Mendel's pea mutants and allow them to integrate their understanding of a visible phenotype with their knowledge about the underlying molecular mechanisms that regulate the phenotype. Mendel's classic work describing how traits are transmitted

between generations laid the groundwork for our current understanding of genetics. His description of transmission genetics preceded modern conceptions of the molecular basis of these phenomena by decades and beautifully illustrates the awesome power of genetics to provide biological insights without the need to know anything about molecular mechanisms. Mendel used seven visible phenotypes, each controlled by a single gene, in his seminal work: plant height (*Le*), seed shape (*R*), seed and flower color (*A*), cotyledon color (*I*), fruit shape (*V*), fruit color (*Gp*), and inflorescence architecture (*Fa*) (Lester et al., 1997; Mendel, 1865). Several of the genes responsible for these phenotypes including *R*, *I*, and *Le* have since been cloned and characterized (Armstead et al., 2007; Bhattacharyya et al., 1990; Bhattacharyya et al., 1993; Lester et al., 1997; Martin et al., 1997; Sato et al., 2007).

Although Mendel's work and molecular genetics are often taught together in introductory biology courses, the mode of action of the genes that underlie Mendel's phenotypes is not always addressed in an integrated manner that explicitly links Mendel's work with modern molecular genetics. Mendel's pea phenotypes provide a great opportunity to connect genotypes to phenotypes because the causal genes have been identified and can be used to illuminate fundamental concepts in biology. Consider the *Rugosus* (*R*) gene, which is responsible for the "difference in the form of the ripe seeds" (Mendel, 1865) and results in wrinkled seeds when mutated (Bhattacharyya et al., 1990; Bhattacharyya et al., 1993). During seed maturation, peas accumulate large amounts of polymerized sugars in the form of amylopectin, a branched form of starch. This starch functions as a food reserve used to drive the rapid initial growth of germinating seedlings. *R* encodes a starch-branching enzyme which, when mutated, results in decreased levels of amylopectin and increased amylose (unbranched starch) and sucrose levels. Increased sugar levels lead to higher osmotic pressure in the cells causing the developing seeds to swell. When they dry at maturity, *r* seeds shrink more than *R* seeds resulting in a wrinkled as opposed to a smooth morphology (Bhattacharyya et al., 1990; Bhattacharyya et al., 1993). This mechanistic understanding of seed shape phenotypes can be used to link discussions of Mendelian genetics to fundamental biological concepts including osmosis, turgor pressure, the structure and properties of biological polymers, and the activity of starch-modifying enzymes. Similarly, the *I* gene, which is responsible for cotyledon color (green vs. yellow) encodes an enzyme required for chlorophyll catabolism (Armstead et al., 2007; Hortensteiner, 2009). The connection between the degradation of a photosynthetic pigment and a change in tissue color clearly illustrates the light absorbing property of pigments. It can also be used a starting point for

discussing the molecular basis of leaf senescence and nutrient remobilization of agriculturally important stay-green traits, and of the conservation of genes and phenotypes between species.

The laboratory exercise we designed requires students to collect, analyze and interpret multiple lines of convergent evidence using a combination of molecular and physiological techniques. We chose to base the experiment on *Le*, a gibberellic acid (GA) biosynthetic enzyme that controls stem length (Lester et al., 1997), for several reasons. First, because pea seedlings grow robustly and reliably, mutant *le* (dwarf) phenotypes are clearly visible a week after seed germination. This allows the experiment to be incorporated into courses with a minimal amount of preparation and plant care. Second, the mutant dwarf phenotype can be rescued to full length by a simple foliar application of GA. Similarities exist between this experiment and Beadle and Tatum's (1941) classic experiments which are often discussed in introductory biology courses. Both reveal the relationship between genes and enzymes by using the products of enzymatic reactions to rescue mutant phenotypes. Finally, a large number of molecularly uncharacterized *le* alleles are available from the USDA pea germplasm stock center (<http://www.ars.usda.gov/Main/docs.htm?docid=15144>).

We designed the exercise to be performed over a 3-week interval in a 3-hour laboratory period each week. In the first week, we introduce students to the concept that pea growth depends on the biosynthesis of GA, encoded by the *Le* gene. We distribute seedling controls including wild type (*Le*) and Mendel's mutant peas, in which the dwarf phenotype is caused by a mutation in the biosynthetic pathway (*le*). We also distribute seedlings in which the dwarf phenotype is caused by an unknown mutation. This serves as the experimental condition. Groups of students measure the height and count the number of leaves of each seedling. They then treat these plants with a GA spray. GA treatment during development is known to rescue *le* mutants to full wild-type height. Thus, students are able to conclude whether or not their unknown mutation is in the biosynthetic portion of the GA signaling pathway or is a mutation in a gene required for GA perception or signaling (e.g. the gene that encodes the GA receptor). During the second week the effects of the GA treatment on plant height are measured. Also during the second week, students prepare DNA from each line and set up PCR-based dCAPS genotyping reactions (Neff, et al., 1998) for each of the three lines (wild type, known dwarf and unknown dwarf) to determine if the unknown plants share the same mutation as Mendel's mutants. The PCR products are digested using restriction enzymes between weeks two and three and then analyzed using agarose gel electrophoresis during the third week. There is time built into the

Table 1. Descriptions of Four Learning Goals.

| Learning Goal | Description |
|-------------------------|---|
| Understanding the Lab | Understanding principles and methods of PCR, restriction enzyme digestion and gel electrophoresis and how these techniques can be used in tandem to genotype unknowns |
| Basic Science Literacy | Understanding how molecular methods can be used to address scientific and social issues including those described in the popular media |
| Scientific Practice | Performing laboratory protocols to characterize unknowns, written argumentation in laboratory reports, revising reports following peer-review, and searching and understanding published scientific research literature |
| Working Collaboratively | Undertaking distributed responsibilities in small groups |

third laboratory period to discuss the results of both the phenotypic and genotypic analyses. Finally we ask our students to write up these experiments as a laboratory report in the style of a scientific research paper.

Hallmarks of authentic scientific inquiry include *understanding the lab* in terms of principles & methods, *basic science literacy* such as how molecular methods can be used to address scientific and social issues, *scientific practices* including searching and understanding scientific literature, and *working collaboratively* i.e. undertaking distributed responsibilities in small groups (Table 1; NRC, 2000).

Opportunities for authentic practice can engender a sense of contribution and belonging in a community and inspire students to further participation beyond the classroom (Lave and Wenger, 1991). Inquiry-based science education has been shown to improve students' confidence in their understanding of and capacity to use scientific concepts, including students who do not envision themselves as future scientists (Kudish, 2009; Roth & Lee, 2004). In this paper we present several lines of data to evince the success of the lab. These include student self-reported "pain vs. gain" to compare the laboratory exercise to others in the course and to an earlier incarnation of this exercise that did not include unknowns or genotyping. We also assessed changes in students' confidence in four proficiencies students acquire through participation in inquiry-based laboratories, as described in the literature (Table 1.)

MATERIALS & METHODS

Course and Institutional Context

This research was performed at Swarthmore College as part of a semester-long team-taught introductory cellular and molecular biology course (Bio1). The course is designed for both biology pre-majors and non-majors and every year 70-80% of the ~120 students enrolled in the course are freshmen. Students attend lecture *en masse* and are divided into five or six laboratory sections of up to 24 students each. Four faculty members lecture in the course on topics including both Mendelian and molecular genetics. A faculty member and a professional laboratory instructor or a pair of laboratory instructors teach each laboratory section and are

assisted by an undergraduate student teaching assistant.

Description of the Lab: Mendel's Mutant Peas

The laboratory handout that we provide to our students and detailed instructors notes are provided as online supplemental materials at the following URL: <http://www.swarthmore.edu/biology/mendels-mutant-peas-i-iii>

Assessments

We collected three lines of evidence to evaluate the success of the laboratory in improving students' motivation and confidence in their learning: self-reported "pain vs. gain," degree of motivation associated with characterizing a previously unknown mutation (as opposed to recapitulating an expected result) and changes in confidence based on four learning goals described in Table 1. Data collection methods included pre- and post-laboratory surveys (2009) and end-of-term surveys (2007 and 2009). We used the following methods to generate these data.

Pain vs. gain: We calculated student ratings of the "pain" versus the "gain" associated with the laboratory in end-of-term surveys (Aronson & Silveira, 2009). To differentiate between the outcomes of teaching with (2009) and without (2007) the genotyping component we considered differences between absolute pain and gain scores within and between semesters. We also rank-ordered all of the exercises in each semester based on gain/pain ratios to compare the pea laboratory with the other laboratories taught in each semester. We assumed that higher grades might lead to more positive self-reports regardless of the incorporation of the new inquiry-based elements. Thus, to eliminate grades as a possible confounding variable for positive student response, we compared mean writing assignment scores associated with this laboratory across both semesters.

Motivational effects of characterizing true unknowns: We administered pre- and post-laboratory surveys before the start of the first week and at the end of the last week of semester in 2009. In the post-laboratory survey, we asked students to rate the motivational effects of characterizing true unknowns vs. pre-determined outcomes.

Changes in confidence based on four learning goals: Using pre-laboratory and post-laboratory

Table 2. Mean Pain and Gain ratings (0-4 scale) for all Fall 2007 Bio 1 labs (n=109).

| Laboratory Project | Gain | Pain | Difference |
|---------------------------------------|------|------|------------|
| Antibiotics | 2.66 | 1.10 | 1.55 |
| Mendel's Mutant Peas (traditional) | 2.72 | 1.25 | 1.48 |
| Structure and Function of Plasmid DNA | 2.76 | 1.34 | 1.42 |
| DNA/PCR | 2.76 | 1.40 | 1.36 |
| Biotechnology & Society Presentations | 2.62 | 1.46 | 1.16 |
| Cell Diversity | 1.84 | 0.70 | 1.13 |
| Earthworm Action Potentials | 2.42 | 2.22 | 0.20 |

surveys we calculated differences in student responses to 15 matched questions to assess effects on student confidence in understanding the lab, basic science literacy, scientific practices and working collaboratively (Table 1). We used 2-tailed paired t-tests to test for significance.

RESULTS:

Before 2009, this laboratory consisted solely of the phenotypic analysis of the effects of GA treatment (weeks 1 and 2 of the current lab) using previously characterized *le* mutants. In order to integrate molecular and phenotypic analyses into a single laboratory exercise we developed a dCAPS-based genotyping protocol that was included for the first time in 2009. We also wanted to infuse the laboratory with elements of inquiry so we expanded the laboratory to include the characterization of 'unknowns,' lines of short plants that are presumptive *le* mutants.

In post-laboratory surveys, students rated the pea laboratory as having the highest absolute gain and the greatest gain to pain difference of any of the eight labs taught in the course in the fall of 2009.

Compared with ratings of the traditional fall 2007 lab (Table 2), the inquiry-infused 2009 laboratory had a higher gain score, greater gain-pain differences, and rose from second to first in same-semester rankings against other labs (Table 3).

Mean laboratory report scores were similar between fall 2007 (Mean=85.82, SD=7.81, n=109) and fall 2009 (Mean=85.30, SD=6.77, n=114), suggesting that higher grades are unlikely to be a confounding variable for increased ratings in the end of the semester survey. Students rated the

characterization of true unknowns as somewhat to very motivating with very motivating being the highest on a 4-point scale, possibly implicating the inclusion of unknowns as a factor in the high gain ratings for this laboratory (Table 4).

Our assessment revealed significant increases in student confidence following participation in the inquiry-based laboratory (Figure 1). Of the four learning goals, students reported the greatest increases in confidence in understanding the lab, followed by basic science literacy. Student confidence in their ability to participate in certain scientific practices also improved. These included formulating a testable hypothesis, designing a laboratory experiment, performing experiments independently and forming and supporting arguments in the discussion section of a laboratory report. By contrast, their confidence in other scientific practices, specifically those involving primary literature, did not significantly improve. Additionally, students were more confident working collaboratively than independently.

DISCUSSION

We endeavored to create an introductory laboratory that connects phenotypes and genotypes and ties together Mendel's traits with an understanding of the molecular mechanisms that regulate them. One of our goals was maintaining the practical features of traditional labs in a large course while at the same time incorporating those features of inquiry-based labs that enhance student learning and motivation. Three convergent lines of evidence suggest that this laboratory creates the positive outcomes associated with inquiry-based experiences.

Table 3. Mean Pain and Gain ratings (0-4 scale) for all fall 2009 Bio 1 labs (n=114).

| Laboratory Project | Gain | Pain | Difference |
|--|------|------|------------|
| Mendel's Mutant Peas I, II & III (inquiry based lab) | 3.22 | 0.94 | 2.28 |
| Regulation of Gene Expression I & II | 2.84 | 0.94 | 1.90 |
| An Experiment in <i>Drosophila</i> | 3.02 | 1.15 | 1.87 |
| A Virtual Introduction to Mendelian Genetics | 2.22 | 0.50 | 1.73 |
| Neurobiology I & II | 2.65 | 1.35 | 1.30 |
| Human Genetics | 2.18 | 0.97 | 1.20 |
| Cell Diversity & Life and Death in Bio | 1.72 | 0.74 | 0.98 |
| Bioinformatics | 1.74 | 0.89 | 0.85 |

Table 4. Post-lab survey responses indicating motivation derived from characterizing true unknowns as wild-type vs. mutant (n=89).

| Number of Students | Description | Rating |
|--------------------|---------------------|--------|
| 1 | Not motivating | 0 |
| 6 | A little motivating | 1 |
| 50 | Somewhat motivating | 2 |
| 32 | Very motivating | 3 |
| 89 | Mean rating | 2.27 |

Firstly, students reported more gain and less pain than in a previous semester, prior to inclusion of true unknowns and genotyping. Secondly, they reported more gain and less pain compared with ratings of non-inquiry labs taught in the same semester. Thirdly, these students were motivated by the opportunity to characterize true unknowns and gained confidence in their capacities to understand and participate in certain scientific practices. These included formulating a testable hypothesis, designing a laboratory experiment, performing experiments

undergraduates' confidence in navigating primary scientific literature (Kozeracki, et al., 2006).

Students were more confident working collaboratively than independently. However, their confidence in working collaboratively did not significantly change after participation in the laboratory. We speculate this indicates that our students were already accustomed to working with partners or other small groups in previous courses or contexts e.g. during primary and secondary schooling or in traditional labs earlier in the semester.

Overall, our findings support the conclusion that infusion of inquiry elements into an otherwise traditional introductory biology laboratory for a mix of biology pre-majors and non-majors results in increased student motivation and confidence in understanding scientific concepts and undertaking scientific practices. These outcomes support recent quasi-experimental studies showing gains in comprehension, enjoyment, skills and attitudes toward science for biology majors (Rissing & Cogan, 2009) and non-biology majors (Lord & Orkwisezewski, 2006.)

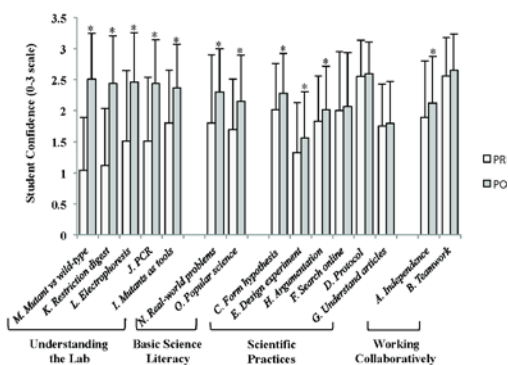


Fig. 1. Mean pre-laboratory and post-laboratory student confidence on a scale from 0-3 (0=not at all, 1=a little, 2=somewhat, 3=very motivating) in 15 items (A-O) representing four learning goals (n=89). *p < 0.01 (2-tailed paired t-test)

independently and forming and supporting arguments in the discussion section of a laboratory report.

Students' confidence in other scientific practices, specifically those involving primary literature, did not significantly improve. These included the ability to search online for scientific research articles and understand scientific research articles. During the laboratory, instructors modeled an online primary literature search for the introductory students on a projection screen and we required primary literature citations to support arguments in students' laboratory reports. However, we did not guide students through their own literature searches. Our results suggest that a demonstration is insufficient to increase student confidence in this practice. This finding is consistent with pedagogical literature describing the need for and efficacy of intensive scaffolding to increase

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