Undergraduates Choose Your Own Adventure: Inquiry-Based Research in Plant Biology and Developmental Biology Classes

Georgette Briggs[1*§], Chris J. Meyer[2*§], Shelley Hepworth[3] and Steven P. Chatfield[4*]

[1]The University of the West Indies, Department of Life Sciences, St. Augustine, Trinidad and Tobago
[2]University of Guelph, Department of Molecular & Cellular Biology, 50 Stone Rd E, Guelph Ontario N1G 2W1 CAN
[3]Carleton University, Department of Biology, 1125 Colonel By Dr, Ottawa ON K1S 5B6 CAN
[4]University of Toronto Mississauga, Biology Department, 3359 Mississauga Rd, Mississauga Ontario L5L 1C6 CAN
*Presenters at 2019 ABLE Ottawa CAN
§Contributed equally to the publication
 (**georgette.briggs@sta.uwi.edu; cmeyer02@uoguelph.ca; steven.chatfield@utoronto.ca**)

We have developed inquiry-based courses in plant and developmental biology using readily available resources for the model plant *Arabidopsis thaliana*. Students "choose-their-own-adventure" in a format that develops critical thinking, experimental design and scientific communication as they learn and integrate core biological concepts. Students design genetic screens to identify mutants affecting a specific developmental process, undertake special skill-building labs, and conduct their own experiments to characterize the mutants. They also choose 'virtual' experiments to perform and must rationalize their experimental choices. Students organize data from their real and virtual experiments to construct figures for their final presentation. They experience how a biological question can be broken down into component parts that can be addressed with different experiments and techniques. We provide here examples of a 'virtual' exercise and a skill-building lab.

Keywords: plant, botany, development, experimental design, genetics, inquiry-based learning

Introduction

Inquiry-based learning is a type of active learning approach that can incorporate many structured engagement practices such as experimental design and investigation, problem-solving, data interpretation, predictions and discussions amongst students (p.23 NRC, 1996). This approach brings with it a change in typical classroom teaching methods, moving away from the dyadic lecture, towards a focus on student-centered learning. This ultimately places more responsibility on the student, requiring their engagement in the learning process at hand. Inquiry-based learning is not a novel approach in science, however over the last two decades there has been a resurgence in the use of these approaches in the classroom investigation of science (Magee and Meier, 2011). Deslaurier and colleagues had previously investigated these effects in a large science class (Deslaurier, 2011) and more recently demonstrated that these active learning approaches provide benefits to student learning, student retention of knowledge and student development of scientific thinking and deduction (Deslaurier et al., 2019). In the current paper, we demonstrate the use of inquiry-based learning in our course design, where students in a developmental biology class design experiments, identify and characterize mutants, and choose their own approach to rationalizing and problem-solving.

Course Design and Re-structure for Inquiry-based Components

The inclusion of inquiry-based approaches into biology courses can sometimes be accomplished by the integration of research components into the original course design. However, in most cases, restructuring an existing course or developing a new one is the more feasible option. One obvious consideration in developing or restructuring a course is anticipating the knowledge, skills and techniques the students will need to complete their experiments and/or long-term projects. Then taking into account the level taught and prerequisite courses, a course designer needs to decide how the students will be given the opportunity to obtain the assets they do not already possess as they progress within the course (e.g. through skill-building labs, seminars, personal/group research, or even 'flipped' classroom approaches).

The relevant course structure chosen will also likely be influenced by several other factors, some of which may rely on budgetary limitations and departmental buy-in. Class sizes will also influence the approach and may in turn impact budget. For example, a department may not support as many lab hours or resources if student numbers are low. Conversely, it is often easier to support students in small class situations with adequate advice and feedback to help them navigate an open-ended experimental landscape.

At the core of the inquiry-based approach is the engagement of students in meaningful research experiences. In class this can occur through literature research, the development of questions, hypotheses and experimental plans, and in the lab through active engagement in the scientific process. Students are allowed to devise their own approaches to addressing questions and determine the steps they will take to answering these questions following an open-inquiry format. The use of *Arabidopsis thaliana* mutants (available for almost every gene in this model plant genome) and genetic pathway analysis provide an endless resource for discovery science and hypothesis testing.

Our Courses

At the 2019 ABLE meeting in Ottawa, we presented activities from our 3rd year plant development courses. Each of our courses utilize the extensive (and affordable) genetic resources available in the model plant system *Arabidopsis*, and challenge students with an open-ended inquiry-based format. Both the resources and elements of the approach could be successfully utilized by courses in other topics at different levels, such as genetics or introductory botany.

Arabidopsis stocks we utilize were originally obtained as seed stocks (USD$10/stock or free for grades K-12) from the Arabidopsis Biological Resource Center (ABRC, https://abrc.osu.edu/educators). Once obtained, it is relatively easy to grow and bulk your own inexhaustible supplies of these seeds. Each seed stock we use typically

represents a single mutant allele that causes an obvious and easy-to-score developmental phenotype in both the seedling and adult plant. The 'control' to which these mutants are compared must also be obtained and grown. It is referred to as 'wild type', which represents the original genotype in which the respective mutations were generated.

Broadly, our course labs begin in one of two ways. In the first approach we ask our students to choose a developmental process/phenomenon (e.g. root growth, auxin regulation of development, leaf development), then have them design a genetic screen that will allow the identification of mutants affecting these aspects of development. Once the students have identified one or more mutants, they go on to characterize the defects and attempt to draw mechanistic conclusions about what is causing them. In the second approach we provide students with an unknown mutant and ask them to characterize and assay it to work out how it differs from wild type and what gene(s) might be affected by the mutation. As each course progresses, the students choose or design experiments to test their mutants and accumulate data that they must interpret. At the end of the course students have essentially completed a research project and must organize and present their data to support their conclusions.

From the student perspective, the first approach can appear completely open-ended. A real mutant screen would comprise a population of individuals in which random mutations had been induced. Even if the density of these mutations were 1000 hits per genome, a very large population (and lots of space, resources and time) would be needed to ensure that on average each gene was likely affected by a loss or gain of function mutation somewhere in the population and that each individual in the population was screened adequately. Additionally, with this approach we could not be sure that a student group would find something relevant and these screens typically reveal more false positives than genuine targets. So, to limit the resources and time expended and to enable the course to predict and anticipate what each group will need (e.g. virtual experimental data), the screen is not an open-ended exercise. Each screen consists of ~2000 wild type seeds 'spiked' with a selection of mutant seed, typically two well-characterized relevant mutants and three interesting 'red herrings' (~10 seeds of each). The 'virtual data' provided to the students during the course for their mutants was previously generated by project students and/or reverse engineered from published data.

In both approaches students typically examine their mutant at the organismal, organ, tissue and cellular levels, comparing and contrasting it with wild type. This requires a working knowledge of plant anatomy and may usefully draw on microscopy and histology skills. To help them monitor plant growth and/or generate quantitative

data from images and videos, our students also learn image analysis techniques. Students first perform a lab activity designed to familiarize them with the use of online genomic resources that will also support their research objectives. To furnish them with the required technical knowledge, our courses also include "skill-building" labs. For example, two labs in the first half of a course couple investigating root and shoot anatomy with the acquisition of basic histology and microscopy.

Overall, the course structure is designed to mirror that of real studies wherein developmental mutants are identified, characterized, and the genes they affect determined. Typically, these types of research projects would include findings from techniques that are very difficult to implement in an undergraduate lab due to time-required (e.g. genetic crosses), cost (e.g. genetic mapping with high-throughput sequencing) or equipment needs (e.g. Scanning Electron Microscopy). Instead of bypassing/ignoring these techniques, we use 'virtual experiments' to give students a more complete research experience, a perspective on the capabilities and limitations of these techniques, and to help them understand how they can be integrated to answer sub-questions of a broader research project. An example of the format of data provided to students as the outcome of one of these virtual experiments is provided in Appendix C. In these virtual experiments, students are provided with the data these techniques would generate if applied to their specific mutant. The students must then organize, analyze and present this data, including it in the Results section of their final presentations. Often

students also 'choose-their-own-adventure' by selecting which virtual experiments they will perform to support their long-term project. They must also rationalize and explain their choicesand why they opted for one experiment over another. To equip them with the understanding they need to make these choices, some of the early course seminars focus on the techniques used in the field of developmental biology, their application and limitations.

To help readers relate our course components to the inquiry-based structure, activities and overall learning objectives, we have provided a table (Table 1, Appendix A) that summarizes chronologically how the content is delivered to students.

At the ABLE meeting in Ottawa our registrants participated in three separate activities from our collective courses. Two of these activities were essentially skill building labs, the first of which focused on using Raspberry Pi computers to analyze images and videos of plant growth as part of the student projects to study their mutants. In the second activity students would learn to utilize online databases and data visualization tools to make discoveries related to an 'unknown' protein sequence. In our third activity students receive data on genetic crosses they elected to perform 'virtually'. Students organize this data and use it to draw conclusions about their mutants (Appendix C). Herein we present the skill-building lab utilizing online databases to answer research questions centered on the 'discovery' of an unknown protein.

Student Outline

Objectives:

- Learn how online genomic and data visualization resources can be used to answer biology research questions and make novel discoveries.
- Experience how a research project can be broken down into smaller questions that can be answered with experiments or by finding and analyzing publicly available data.
- Rationalize and understand why each experiment was proposed and performed.
- Postulate what a researcher can learn from proposed experiments.

Introduction

Greetings developmental biologists, welcome to the lab. Today you are a virtual grad student. Your new PI (Principal Investigator (Prof)) in Canada has a collaborator in South America and they have joint funding for a project in *Theobroma cacao*. Specifically, they are interested in developmental responses (e.g. leaf-shedding) to biotic stresses (such as insect herbivores or disease). Proteomic work in South America has identified a protein found in the shoot apical region that is also found in abscission zones. As you know abscission zones are specialized regions at the junction between lateral organs (e.g. leaves, fruit, flowers) and the stem. Environmental changes (e.g. drought) and biotic stresses (e.g. pest infestations) may initiate changes in the abscission zone that lead to the controlled shedding of the lateral organs.

The sequence of this intriguing protein can be seen below. The amino acids in the polypeptide chain are denoted by their letter codes.

MSSFEESLRSLSLDYLNLLINGQAFSDVTFSVEGRLVHAHRCILAARSLFFRKFFCGPDPPSGLDPVGSRMNPASAAA
AGSRPGVIPVNSVGYEVFLLLLQFLYSGQVSIVPQKHEPRPNCSERACWHTHCTSAVDLALETLAAARYFGVEQLAL
LTQKQLASMVEKASIEDVMKVLIASRKQDMHQLWTTCSHLVAKSGLPPEVLAKHLPIDVVAKIEELRLKSSLARRP
LITHHHHHHDLTSTADLEDQKIRRMRRALDSSDVELVKLMVMGEGLNLDEALALHYAVENCSREVVKTLLELGAA
DVNYRAGPAGKTPLHIAAEMVSPDMVAVLLDHHADPNVRTVDGVTPLDILRTLTSDFLFKGAVPGLTHIEPNKLRL
CLELVQSAALVISREEGSANAPTSTAIYPPMSDEHNSSSSGSNLATLNLDSRLVYLNLGATGSTQMGSRMEGDDDSS
HNSQREAMNRHDPTMYHHSHDF

Methods and Data Collection

Your PI has asked you to find out more about the likely function of this protein. They want you to find out what is known about its role in development and stress responses in a better characterized dicot model system (*Arabidopsis*). They would also like to know if it shares a genetic pathway or protein-protein interactions with any of the other proteins they have managed to isolate from the same regions of *Theobroma cacao* plants. Lastly, they would like to connect the dots and find out if it is involved in any signaling/response pathways that directly mediate resistance to pests or disease.

Questions you can ask that might guide your investigations could include:

- What are the closest homologues of this protein identified in other species?
- What are the closest homologues of this protein in *Arabidopsis*?
- What type of protein is it, and where is it localized in cells?
- Where is this gene expressed in other plants?
- How is plant development changed when you knock these genes out?
- How is plant development changed when you over-express these genes?
- What resources (mutants, over-expression lines, transcriptional reporters, etc.) are available for me to study the role of this gene in pest/disease resistance or abscission in *Arabidopsis*?
- What is known about the genetic interactions this gene participates in?
- How is the expression of other genes affected by this gene?
- What is known about potential protein-protein interactions?
- ….and so on.

You are going to answer some of the above questions in the next 3 hours. Bonus: You will not need to know any code or learn a programming language (yay again). This lab will take you through the steps using the online resources at your disposal. These resources are tools commonly used by plant science (and some more generally in biology) researchers (academic and industrial) and you will often be expected to be familiar with them and their use.

If this sounds a little daunting, fear not (double bonus) you get help. Your PI has taken on another student to help with this project, **i.e. you work in pairs (3's max) for this lab.**

The lab exercise is separated into steps relating to those important questions. **At the end of each step (question section) talk to the TA/Prof to confirm that you have completed it successfully and discuss the implications of your discoveries before you move to the next step. Feel free to ask questions at any other point too.**

By the end of this lab exercise, you will have used data other researchers have made public to answer a bunch of your own research questions and come up with some novel targets for your future research. As you go you can write and/or copy and paste your results into this word document, which you will hand in at the end. Also, think about how you might use the resources you are exploring to enhance your own research project. How could some of these tools be used to support a research project centered on one of the mutants you have discovered?

Use the Worksheet provided **(Appendix B)** to help you answer research questions related to this unknown protein.

Materials

This activity requires computers connected to the internet (typically one per student pair). All but one of the web-resources it utilizes is free. The resources all appear to function fully in Firefox, Chrome and Microsoft Edge browsers, and on Mac or PC. The Arabidopsis Information Resource (TAIR) (Berardini *et al*, 2015) may permit some limited use, but may require a subscription after a period, or to use certain pages (https://www.arabidopsis.org/doc/about/tair_subscriptions/413).

A single academic subscription costs $10.80 USD per month. A free month trial is available at the institutional level. There are various levels of academic institutional use that begin at $1090 USD per year. This lowest usage level should be sufficient if your institution does not already have a subscription (most larger universities seem to have one). We suggest trying the activity on your lab computers to establish if it involves access to pages that are currently behind a subscription wall, or if your organization does/does not have a subscription. Students generally have a Microsoft Word version of the activity document open on the same computer they perform the activity to fill in the answers as they go.

Notes for the Instructor

Students participating in this plant development course receive seminars focused on the diverse experimental techniques and tools that can be used to investigate developmental questions. These techniques and tools include genetic analysis (e.g. Appendix C), various molecular tools (e.g. reporter fusions and CRISPR), microscopy and histology, physiological measurements and manipulation, growth assays, and even grafting. The class then studies some important research papers and dissects how specific sub-questions of a larger developmental research project were answered experimentally. These exercises are also used to better understand the limitations of many of these techniques and compare different approaches.

The techniques we study include high-throughput sequencing of genomes, transcriptome analysis and proteomic methods. This leads the class to the point where they better understand where the genomic resources available on the internet originate and how they were generated. In this lab the class now works through an example of how these online genomic resources and data visualization tools can be used to support and drive developmental research.

In the activity students do not explore the code, algorithms or computing principles of the online tools they use. One of our goals is to show the students what can be achieved with bioinformatic approaches without focusing on the 'how' of these techniques. We hope that even if this does not succeed in encouraging students to go on to explore bioinformatics and genomics at a deeper level in subsequent courses, then at least they have a better understanding of the value of these approaches.

This lab was also intended to function as a 'skill-building' activity to support students' inquiry-based research efforts in their primary long-term mutant screen and characterization experiment. In a subsequent tutorial, students will use some of the online resources again to try to identify the gene affected in the mutant they have isolated. Once this is achieved some students choose to generate new figures for their final presentation by performing their own independent online research using the tools they have tried in above activity. For example, some will use the BAR ePlant tool (Waese *et al*, 2017) to visualize the expression pattern of their gene.

One issue with the lab is that by bringing together so many online tools and utilizing them in a rigid instructive way, there is a very high chance that an update or change in any of the websites will end up steering students off the 'prescribed' course. The obvious solution to this is to run through it prior to using the activity every term and correct for these changes. A better, but more tricky solution might be to make the exploration of these resources more open and less prescribed. However, when we have tried this we ended up moving through the activity more slowly and students did not get to explore all the online resources we wanted them to experience to support their primary inquiry-based experiment.

This activity is marked to motivate students to complete it, consult with their TA/Prof as they progress and thereby maximize the value of the exercise. A 'TA-key' for the activity is used (available from S. Chatfield), which contains the expected outcomes at each step, plus questions to assess the student's understanding. Mistakes in procedure are therefore addressed as students move through the activity and check-in with the instructional staff. Most students complete all the sections and provide satisfactory answers at each step. Some marks may be deducted for failures to communicate the answers well or lapses in understanding. Feedback is provided to try to intercept these issues prior to the students' final project presentations.

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Acknowledgments

About the Authors

Dr. Georgette Briggs (Presenter) has been a Lecturer at The University of the West Indies (UWI) since August, 2008. She earned a MEd in Curriculum (2013) from The UWI and has since been heavily involved in curriculum development, course design and the Scholarship of Teaching and Learning (SoTL). She currently teaches 2nd year courses in Cell & Developmental Biology and Molecular Biology Techniques, and a final year course in Plant Development at the St. Augustine campus in Trinidad & Tobago. Her research in science education involves the use of technology in the classroom and the integration of research in the undergraduate experience.

Dr. Chris J. Meyer (Presenter) received a PhD in Biology from the University of Waterloo in 2010. His involvement with undergraduate teaching was honed at the Universities of Waterloo, Wilfrid Laurier and Brock. Since 2013, he has been an Undergraduate Laboratory Coordinator at the University of Guelph, where he designs and delivers botany lab courses for 2nd - 4th year students.

Dr. Shelley Hepworth (Contributor) is a Professor in the Department of Biology at Carleton University where she conducts research in plant biology and teaches 3rd year courses in Cell & Developmental Biology. Undergraduates are incorporated into her research program at many levels. She received her Ph.D. from the University of Toronto in 1998.

Dr. Steven Chatfield (Presenter) is an Assistant Professor Teaching Stream with the Department of Biology at the University of Toronto Mississauga where he teaches/coordinates 2nd & 3rd year Plant Biology, 3rd year Molecular & Cellular Biology Labs, a 4th year Internship Program, a 1st year Scholars course and acts as a faculty adviser with the 4th year Advanced Interdisciplinary Labs course. Prior to 2014 he was a sessional lecturer, and post-doctoral researcher at Carleton University (Biology), U of T St George Campus (Cell & Systems Biology) and Guelph (Plant Agriculture).

Appendix A: BIO353 Plant Development

Table 1. Chronological Course Components, Activities and Key Objectives**.**

Course Component	Activities	Key Objectives
Seminar 1	- Introduction & overview of course. - Overview roles of hormones in plant development. - Forward & reverse genetics.	- Students acquire essential background knowledge needed to design mutant screen. - Ensure clear definitions of gene, mutation, mutant, and wild type, and how they can be used to study development. - Explore pros/cons of forward vs reverse genetics.
Tutorial 1	- Set up research groups (~4 students). - Debate and select developmental process of interest. - Formulate question mutant screen will address.	- Hand-in of initial goals for screen at end of class. - Students initiate literature research. - Groups to divide tasks fairly and assign responsibilities.
Lab 1	- Design of Mutant Screen. - Students provided with limitations (e.g. space) and constraints (e.g. age of plants) for their mutant screen. - Literature searches by students to guide experimental design proposal. - Feedback from mentors (Prof & TA) and peers on utility of their approach.	- Student groups produce workable screens that will identify the mutants we will include in their screening population and fit within the space, resource and time constraints of the course. - Student completed screen design worksheet guides technical support for screen (e.g. preparation of media).
Seminar 2	- The root and shoot meristem. - Techniques for discovery in developmental biology.	- Build on key concepts in plant development introduced in second year. - Provide more necessary background for students to make appropriate choices in their long-term experiment to screen for and characterize mutants.
Tutorial 2	- Introduction to tissue culture. - Setting up the screen	- Student groups set up their screens. 15 petri plates per screen
Lab 2: The shoot	Students learn basic microscopy and histology, and images captured with eyepiece cameras to answer questions about the structure of the shoot apical meristem.	**Skill & knowledge building:** Learn microscopy, histology and photography techniques that can be applied to the students' long-term mutant research project.
Seminar 3	Phyllotaxy and the development of leaves.	- Stages of lateral organ development and contribution of cell division and expansion to it. - The role of phytohormones and specific genes in regulating phyllotaxy and leaf shape.
Tutorial 3	First **virtual experiment.** Students choose between five virtual experiments that could be usefully performed	Demonstrate an understanding of the research project structure by selecting an appropriate virtual experiment to

	immediately after they identify their mutant. ● These experiments involve genetic crosses with wild type and other mutants	perform at this stage in the project and explaining the choice. ● Constructing and presenting an argument.
Lab 3	Students use basic microscopy and histology, and images captured with eyepiece cameras to answer questions about the structure of the root apical meristem and root architecture.	**Skill & knowledge building:** Learn additional histology techniques and knowledge of RAM structure that can be applied to the students' long-term mutant research project.
Seminar 4	The root apical meristem and root architecture.	● Organization of tissues and cell types in the root. ● Genetic and hormonal regulation of root growth and lateral root initiation.
Tutorial 4	Data from their 1st **virtual experiments** (genetic crosses) are given to students and analyzed in class. This corresponds to **Activity described in Appendix C.**	● Revise use of a Punnet square. ● Determine if each mutant phenotype is caused by changes to a single/multiple genes. ● Determine if the mutation is dominant, semi-dominant or recessive. ● Determine if there are maternal effects on the phenotype. ● Determine if multiple mutants have lesions in the same gene.
Lab 4	Online research: Using (other peoples) BIG DATA. Activity described in body text of manuscript	**Skill & knowledge building:** Learn how online genomic resources can be used to make discoveries, answer questions or support findings in developmental biology.
Seminar 5	Lessons from a Key Paper: Nakajima et al 2001.	● Positional signals in plant development. ● Transcription factors and differentiation. ● Movement of transcription factors. ● How each discovery was made/supported experimentally.
Tutorial 5	Second batch of **virtual experiments.** Students are given an excel workbook containing data from three different experimental assays of their mutant.	● Assay data is specific for each group and mutant. ● Broadly the students will organize, analyze and ultimately present this data in their final presentation. In doing so they may answer questions about the hormone responses of the mutant and quantify developmental abnormalities.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and perform assays to support their long-term project.	Student designed assays, student observations and measurements augment to virtual data and findings in final project presentation
Seminar 6	Lessons from a mutant screen in a parasitic plant: Cui et al 2015	● Forward genetics approaches a developmental question without certain biases. ● Developing a novel screen. ● Parallels between parasitic structures and normal root development. ● How each discovery was made/supported experimentally.
Tutorial 6	Image/video analysis.	**Skill & knowledge building:** ● Learn to use Raspberry Pi computers to image and video plant growth.

		or
		● Learn how to analyze images and video in ImageJ to provide quantitative data on plant growth and development.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and perform assays to support their long-term project.	Student designed assays, student observations and measurements augment to virtual data and findings in final project presentation.
Seminar 7	Flowers and fruit.	● Stages of flower and fruit development. ● Genetic control of flower and whorl identity.
Tutorial 7	High-Throughput Sequencing mapping. The final **virtual experiment** in which students try to identify the gene affected in their mutant.	● Students construct scientific arguments for which gene or genes in a given region of a chromosome might be responsible for the phenotype they have characterized. ● In many cases these arguments cannot reasonably limit the choice to a single gene without extensive literature research and use of online databases.
Lab 5	Students use basic microscopy and histology, and images captured with eyepiece cameras to answer questions about the structure of the inflorescences, flowers and fruit.	● Investigate the diversity of flower and fruit structure. ● Learn how flowers give rise to fruits developmentally. ● Some of the skills and knowledge learned may be used to investigate the floral/fruit structure of the students' now mature mutant plants.
Seminar 8	Auxin signaling: It does everything.	Role of auxin in patterning in the embryo and in the initiation of new organs.
Tutorial 8	Term-test 'redemption'. Each student is given the opportunity to reattempt the question they lost most marks on in the exam to recover those marks.	The entire class are incentivized to reexamine all the problematic concepts and develop a better understanding of them.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and perform assays to support their long-term project.	Student designed assays, student observations and measurements augment to virtual data and findings in final project presentation.
Seminar 9	Cytokinin signaling: Story of a quintuple mutant.	The role of cytokinin in differentiation and patterning.
Tutorial 9	Graphs and figures workshop.	Advice and feedback for students putting together figures for their final presentations.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and perform assays to support their long-term project.	Student designed assays, student observations and measurements augment to virtual data and findings in final project presentation.
Seminar 10	Developmental timing with special reference to the induction of flowering. Includes iBiology video by Rick Amasino.	● Why is timing important? ● The genes controlling the onset of reproductive phase. ● The role of the circadian clock.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and	Student designed assays, student observations and measurements augment to

	perform assays to support their long-term project.	virtual data and findings in final project presentation.
Seminar 11	Cell specific patterning in the leaf.	Genes and signals regulating the position of stomata and trichomes.
Tutorial 10	Presentation workshop	Advice and feedback for students putting together their final presentations.
Lab unstructured	A 3-hour time slot for students to make observations, take measurements and perform assays to support their long-term project. Some students use this time to practice their final presentations.	Student designed assays, student observations and measurements augment to virtual data and findings in final project presentation.
Seminar 12	Bringing it all together: Course revision and Q & A	- Encourage students to explain their understanding of key course concepts. - Integrate course components. - Address misconceptions.
Final Presentations (Tutorial slot)	- Poster presentations can be brought together as a 'mini-conference' using the large screen TVs in the active learning classroom to display each student's research poster. - Peer-review is required and constitutes 30% of the mark.	Hone scientific communication skills (written, oral and graphic).
Final Presentations (Lab slot)	- Oral presentations are performed in the teaching lab. - Peer-review is required and constitutes 30% of the mark.	Hone scientific communication skills (written, oral and graphic).

Note: Seminars and tutorials take place in a dedicated active-learning classroom. Labs occur in a teaching lab, with one standard brightfield compound scope per person and a PC for each pair.

Appendix B: BIO353 Plant Development

Using Online Resources to Answer Research Questions in Plant Development

Question 1: What is known about the domains of this protein and what are the closest homologues of this protein in other species?

Open up the internet browser of your choice, you are going to BLAST! Go to the NCBI BLAST page: http://blast.ncbi.nlm.nih.gov/Blast.cgi

According to the Biology Curriculum Map, you've used NCBI BLAST in other courses. If not, do not fret. You are given some choices based on what you want to BLAST, within each you will find choices of different algorithms to use depending on your query and goal. We have a complete protein sequence to query with and we're going to use "protein blast". Select this from within the five "basic BLAST" options:

nucleotide blast =   Search a nucleotide database using a nucleotide query

protein blast =   **Search protein database using a protein query**

blastx =   Search protein database using a translated nucleotide query

tblastn =   Search translated nucleotide database using a protein query

tblastx =   Search translated nucleotide database with translated nucleotide query

In the web page that appears copy and paste your protein sequence (above) into the box entitled "Enter accession number(s), gi(s), or FASTA sequence(s)". This time around we don't need to choose any further options or different algorithms, just click on the blue "BLAST" button!

a) In the graphic at the top of the results page, the program has displayed two "superfamily domains" in your protein - what are they?

Answer:

b) Underneath the graphic is a list entitled "Sequences producing significant alignments:" What is the entry at the top of the list and which species is it from?

Answer:

c) What species (other than *Theobroma cacao,* which you got the protein from) harbor the proteins with max alignment scores > 900?

Answer:

d) How are these species related to *Theobroma cacao*?

Answer:

Question 2: What are the closest homologues of this protein in Arabidopsis?

Find your way back to the protein BLAST page, where you pasted in your protein sequence. Using the same sequence, change one parameter in the "Choose Search Set" section. Start typing *Arabidopsis thaliana* into the "Organism" box and it will give you options to click on a menu. Select "*Arabidopsis thaliana* (taxid:3702)", then click on BLAST.

a) Which named protein (entry with a 3 letter gene code associated with it, not an 'unknown') has the highest similarity to your protein from *Theobroma*? What is the value of finding homologs in *Arabidopsis*?

Answer:

Question 3: OK, so what do we know about these genes from existing research?

Remember TAIR? Let's return there: www.arabidopsis.org (or via the library). Before you proceed ask your TA/Prof to confirm the gene you are searching with from the end of the last section. In the search box at the top, type in the 4-character gene identifier you found in the last step (leave the search setting on "Gene") and click "search".

a) What's the Locus number (AGI) for this gene?

Answer:

b) Follow the link to the locus page. What is the description for this gene?

Answer:

We can learn a great deal from this page. As you know we can use it to identify and purchase mutant seeds, find publications, genomic and coding sequences, and gene ontologies (GO) for the gene in question. GO terms can give you lots of info about the processes a gene is likely involved in (including development), the subcellular location and the molecular function of the protein. Publications are going to answer a lot of the questions we posed in the introduction, e.g. "What is the loss/gain-of-function phenotype?", and so on. We have limited time though, so we are going to focus on one issue here. You have already found a connection with the abscission zone, now let's look at defense against herbivory which may relate to the "biotic stress" focus of your research project. Do you remember jasmonate/jasmonic acid and methyl jasmonate from BIO203? This hormone group is involved in many processes, but they were first identified as important systemic signaling molecules in the response of plants to wounding and mounting a subsequent defense against attackers. Simply do a search of the webpage (ctrl-F) and see if you can find a mention of "jasmonic acid" and/or "jasmonate."

c) Where did you find these terms? What clues or information does this lead you to?

Answer:

Question 4: Where is the Arabidopsis 'version' of my gene expressed and where is the protein localized?

You will likely get very reliable information answering this question from the publications listed on the locus page. This could include *in-situ* hybridization studies (telling you exactly where the mRNA is on fixed and prepared samples), as well as visible reporters of transcription (e.g. promoter::GFP fusions) and the protein products (promoter::coding sequence-GFP), which may tell you what your gene/gene product are up to in real-time. However, because the published approaches done by other groups might not be completely exhaustive (e.g. testing expression under lots of different circumstances/treatments), and because we want to show you some other cool stuff, we are getting you to try out an eFP browser. An eFP browser gives you a visual output summarizing multiple global expression analysis experiments (hundreds of microarray or RNA-seq experiments) that is easy to interpret quickly without requiring expert knowledge. We'll look at a couple of other tools here too.

a) Copy the locus number for the gene from question 3.

- Go to the BAR… not that bar, this one: http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi

- Click on the "Arabidopsis eFP Browser"

- Paste the locus number into the 'Primary Gene ID' search box, or type in the 3 letter + 1 number gene name, and click search.

The first thing to come up will be the default "Developmental map" which displays the expression levels of your gene across 100s of samples in which global gene expression was measured for 22,000+ genes (this qualifies as "Big Data"). Yellow-Orange-Red = Low-Intermediate-High expression respectively. It should be very visually obvious where your gene was expressed most highly in this view.

b) Where and at what level was the expression of your gene highest, and what was the SD?

Answer:

This is not the maximum level of expression seen for your gene though. Check out the 'tissue specific' map where data from laser micro-dissection or fluorescence-activated cell sorting experiments are shown. Researchers have used these methods to enable them to characterize global gene expression in very small groups of cells.

c) Where can the highest levels of expression be found on this map?

Answer:

Coolaboola, now let's head back to the BAR home page and try out ePlant. Input your gene name abbreviation '_ _ _ _'. When that is loaded click on the 'Cell eFP'.

d) Where is your protein localized in the cell?

Answer:

Question 5: Finding new targets: Are other genes expressed in a similar pattern to my gene and are other proteins known to interact with mine?
Now, go to the "Interaction viewer" and select that. The display will show predicted and experimentally supported interactions between the BOP2 protein and other proteins or DNA. This display only contains 6 candidate proteins and the associated locus numbers are given. If you wanted to you could filter this view based on the level of statistical support for these interactions.

a) Write all the locus numbers down. See if you can find a gene product that is auxin responsive and supply the locus number here.

Answer:

OK, so there are some candidates for interaction with our protein that researchers already know about, let's try to find some new ones! Similar expression patterns of genes might reflect similar regulation of those genes and involvement in the same developmental pathways. Click on "Expression Angler". Then choose "Select by AGI ID". For the view (1) let's try "Tissue Specific", sub-menu "Shoot Apical Meristem", for the AGI ID (2) put in the locus number for your *Arabidopsis* homologue of the *Theobroma* protein, and for limit number of results let's try an r-value cutoff of 0.8-1. Hit 'search'.

b) How many genes made the cutoff?

Answer:

These genes were identified based on the similarity of their expression patterns with your gene of interest in the tissue-specific experiments (literally hundreds of samples characterized by microarrays or RNAseq, by a bunch of different research groups). **Record these genes and list them below**

c) Which of the genes on the list were already identified in the interaction viewer?

Answer:

If you wanted you could also look for genes that are oppositely expressed to your gene of interest, which may be of interest too. They could be down-regulated by your gene or vice-versa. However, let's move on. This data mining has turned up some interesting targets, but we're going to run our own experiment too!

Question 6: Finding new targets, part II: How does expression of my gene affect the expression of other genes?

Global gene expression analysis (GGEA = microarrays or RNAseq) is often used to identify 'genes of interest'. These genes could be direct targets of the gene at the center of your research program. In the context of global expression analysis, 'direct target' means the protein coded by your gene directly binds (with/without partner proteins) to controlling sequences for other genes and affects their transcription.

GGEA analysis can also be used to find various indirect targets: these could include genes where expression is affected by the proteins produced by the direct targets (not so indirect), on through to gene expression changes resulting from changed development (e.g. new/different organs being produced with different patterns of gene expression).

In this experiment (that you will be examining) two genotypes were compared: Wild type and a mutant that has an over-expression phenotype for the gene you have been looking at. Amongst other things, the mutant has altered phyllotaxy and branch angles for flowers and fruit (where pedicels meet the inflorescence stem). This boundary is also a location where the gene is more highly expressed. The nodal tissue was harvested in each genotype, the mRNA extracted, and (two-colour) microarray chips used to examine which genes were differentially expressed in mutant versus wild type nodes.

a) You have been supplied with an excel sheet and all the genes with elevated mRNA levels in the mutant (compared with wild type) are highlighted in green (upper half), and all those with reduced mRNA levels are highlighted in red.

You are going to use another online resource to find out what types of genes are differentially expressed in the mutant. First, a question:

b) Is this experiment more likely to identify direct or indirect targets of your gene?

Answer:

Now open up the excel sheet. This is a heavily processed output from an actual microarray experiment. You have a simple list of genes differentially regulated (up and down) in plants over-expressing BOP2 compared to 'normal' wild type plants. This also comprises, a quantification of this fold-change in gene expression (FC (abs)), a probability value expressing how significant this finding likely is (p (Corr)), a brief gene description and the AGI numbers (ID) for each gene. If you would like to see the steps involved in generating this file from raw microarray data and perhaps the R guidance document that would enable you to duplicate some of those steps feel free to ask me (but this is beyond the content of our course).

c) Go to the ID column and select all of the **green** AGI numbers in it (all the "genes up" in the mutant) and copy them. Now, go to the agriGO web page:

http://systemsbiology.cau.edu.cn/agriGOv2/

Click on the "Analysis" tab, then "Plant", then "Brassicaceae". Leave the 'analysis tool' on the default. Paste your list into the "Query list" box. What we are going to do is find out if there are any 'types' of gene functions that are over-

represented (more common than you would expect by chance) in this list of genes with increased expression in the mutant. This <u>might</u> give us some more ideas about what our gene is doing functionally. To work out if something is over-represented you need to know what the entire pool of possible genes is, in this case all the genes represented on the microarray.

In "3. Select Reference:" click on the pull-down menu and select the "Agilent .. Microarray." option. This is the type of microarray chip used in the experiment. Don't worry about the "advanced options", and just submit your job. The results page gives you two different graphic options (and you are free to check them out) but <u>what we'd like you to copy and paste in the excel workbook you have been given is the table of significant results at the bottom of the page.</u> First click download and this will open up the table in another window. Press CTRL-A (select all), CTRL-C, then paste (right click and choose "use destination formatting") them in the new work sheet called "GO up".

Repeat this process for the genes in red with reduced expression in the mutant, and paste the results table into the "GO down" work sheet.

So you have two sets of GO terms that are significantly over-represented amongst the differentially regulated genes in the nodal regions of the mutant plants. So what does this all mean?

If you were to check out the publications for this mutant (as well as the brief description from TAIR) you would see that it was isolated because of a marked and interesting developmental phenotype. Over-expression also results in a rather extreme developmental phenotype. The genes it is known to interact with genetically also have well characterized developmental roles.

d) <u>Do you have any GO terms on your lists that include the word "Development" and/or "Jasmonic/Jasmonate", what are they?</u>

Answer:

Looks like your experiment has come up with a lot of targets. I wonder if any of these targets were also amongst those you 'discovered' in other peoples' data? Checkout the list of genes you ID'd from expression angling, did any turn up as differentially regulated in your microarray?

<u>Talk through your results with the TA and/or Prof.</u>

Appendix C: Meet My Mutant Activity

Student Outline

Hello again virtual researchers! In this exercise, your group will be given data from experiments offered to you in the last session. Your mission is to process the raw data, analyze it and present the results.

What questions about your mutant does the data help you answer? How will you present your data in such a way as to make the evidence it provides clear to those viewing it? How will you show it is significant (or not)? Ask us questions. It may be very helpful to start by working out the limitations of what you do know about any mutant isolated from a screen before you try to establish what these genetic crosses will tell you.

You can work together and collaborate, work together and argue, but submit individual work. Deadline 1 week from now, submission via Quercus.

Your Results
Back-crossing your mutant to wild type

Mutant 1A x Mutant 1A (weak phenotype)

F1	2,4-D resistant root	155/155
F1	mutant shoot phenotype	155/155

Mutant 1A pollen (weak phenotype) x WT female (40 crosses)

F1	2,4-D resistant root	0/53
F1	mutant shoot phenotype	0/53
F2	2,4-D resistant root	37/141
F2	mutant shoot phenotype	37/141

Mutant 1A female (weak phenotype) x WT pollen (40 crosses)

F1	2,4-D resistant root	0/61
F1	mutant shoot phenotype	0/61
F2	2,4-D resistant root	38/145
F2	mutant shoot phenotype	38/145

You think your screen has also isolated a more severe mutation in the same gene and you perform crosses with that too:

Mutant 1B? x Mutant 1B? (strong phenotype)

F1	2,4-D resistant root	122/122
F1	mutant shoot phenotype	122/122

Mutant 1B? (strong allele) x WT (40 crosses)

F1	2,4-D resistant root	0/22
F1	mutant shoot phenotype	0/22
F2	2,4-D resistant root	82/363
F2	mutant shoot phenotype	82/363

Here as a bonus are some samples of the reciprocal crosses (m=male, f=female) between:

Mutant 1B? (f) x Mutant 1A (m)

| F1 | 2,4-D resistant root | 40/40 |
| F1 | mutant shoot phenotype | 40/40 |

Mutant 1A (f) x Mutant 1B? (m)

| F1 | 2,4-D resistant root | 23/23 |
| F1 | mutant shoot phenotype | 23/23 |

Use the worksheet provided to guide your analysis of these data. The results you obtain should be incorporated into your research project and communicated as part of your final presentation.

Worksheet

Answer the following questions <u>for each set of results (where possible)</u>. We want you to understand why each experiment was done and what a researcher can learn from it. Organize your answers with the following format for each cross: (10 marks).

CROSS: _____

Q1. What questions have you answered with these crosses?

Q2. What conclusions can you draw?

Q3. How can this help your future research with this mutant?

Materials

The data students are given for this activity reflects the expected outcomes for these crosses using the mutant(s) that were added to their screening population, and isolated by them. The data used in this activity is generated from prior student experiments, or published results, or synthesized based on the mutant type. Most of the mutants our students are given are homozygous recessive with relatively straight-forward interpretation. Semi-dominant mutant alleles of *Auxin-Resistant 3* (*axr3-1* and *axr3-3*) are sometimes introduced for students conducting mutant screens to isolate auxin or cytokinin-response mutants, or root development abnormalities (these mutants will appear obviously different from wild type in all these situations). Atypical results like these can also support illustrative comparisons and discussion with the whole class.

Notes for the Instructor

Students perform this activity after they have isolated one or more candidate mutants from their screen. They then perform a tutorial exercise where they select a suitable first experiment to perform from a list of five options. Four of the five choices involve different genetic crosses, anyone of which could generate useful information for a researcher who has just isolated a mutant (affecting an unknown gene). One of the choices (e.g. generate a promoter-GFP reporter fusion for the gene) could also be informative but is not an option until the gene affected by the mutation is known. Students are mostly graded in this exercise for successfully rationalizing and explaining their experimental choices, with only one mark (of ten) allotted for selecting one of the four possible correct answers. The following week they perform this activity, in which they interpret the data that would have been generated by the four correct choices (regardless of the choice they made).

Space and time constraints have restricted most/all student groups to develop long-term experiments that screen for mutants in seedling stage, no more than a week old. So, within 10 days of sowing the seed for their screen students will have identified and begun to characterize their mutants (only mutants with an obvious seedling phenotype are provided to students). If we were to use real genetic crosses students would need to wait an additional 3-4 weeks for their mutants to flower, then perform difficult pollen transfers, wait for seed maturation, sow and wait to score the progeny phenotypes. Additionally, they would have had to have anticipated and sown all the genotypes they needed to perform crosses with so that adult plants were available for pollen transfer. Instead they use the raw crossing data provided by us, which they then interpret.

The version of the activity attached shows that all the progeny of a cross between two of the mutant plants isolated gives 100% mutant progeny, and a back-cross to wild type (the original 'normal' in which the mutant was generated) gives 100% 'normal' in the first generation (F1) and 25% mutant if you let these plants self and make seed (F2). This is consistent with a homozygous recessive mutation (1 mark) in a single gene (1 mark), and the fact that the same proportion is achieved regardless of whether the mutant contributes pollen or egg suggests there is not a disproportionate maternal/paternal impact on the phenotype (1 mark). The subsequent experiments confirm that the second 'strong allele' of the mutant is also homozygous recessive (1 mark) and is indeed affecting the same gene as the first (weaker) allele (1 mark). Five marks are assigned for how these findings are expressed/articulated and what importance/utility students connect with these findings from a research perspective.

The activity is performed in an active learning classroom and during the tutorial we (groups and instructor) typically draw many Punnett squares and discuss our expectations given different mutations and combinations thereof. Generally, most students work to achieving broadly the correct answers for each cross before the end of class. Where marks are lost the majority are part marks that reflect how students have organized and expressed their conclusions and/or communicated the importance of their results.

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