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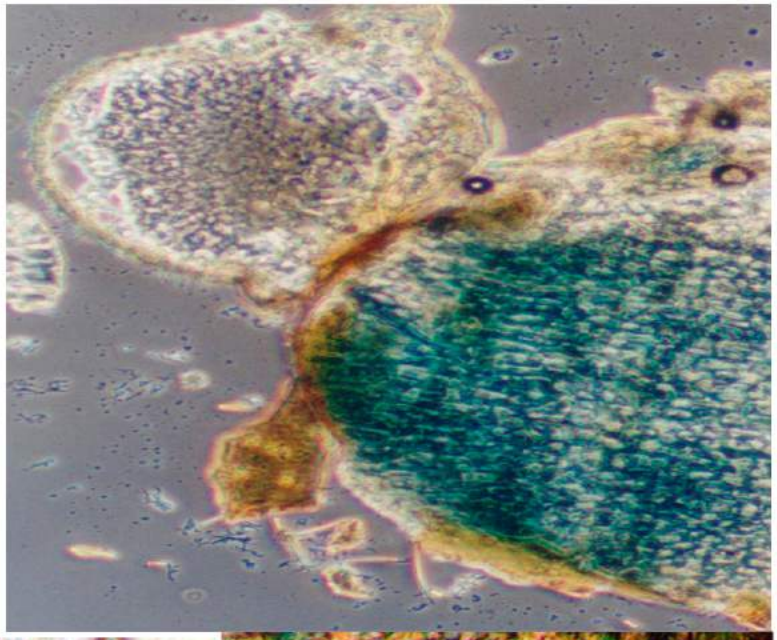
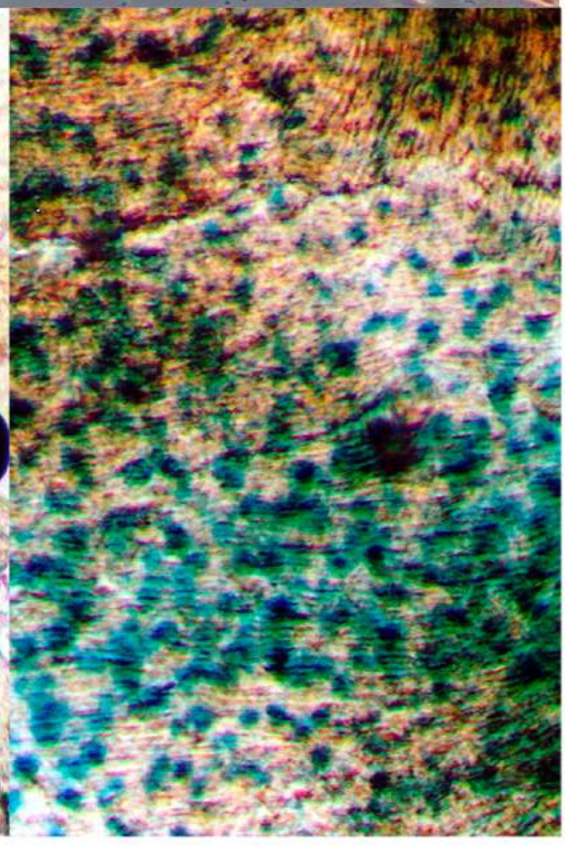
Journal of College Biology Teaching





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Cover image: Composite of micrographs (100X – 400X) of fruit fly embryos *(Drosophila melanogaster) reveal the* expression pattern of the lac Z reporter gene. Note the stained thoracic flight muscles. Courtesy of Beverly Clendening

Articles:

News & Views:

Bioscene: Journal of College Biology Teaching

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April 1, 2002 for the May 2002 Issue **July 1, 2002 for the August 2002 Issue**

An Advanced Molecular Techniques Laboratory Course Using *Drosophila melanogaster*

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ABSTRACT: This advanced molecular biology laboratory course uses a project approach to learning and incorporates an independent research component. The students use enhancer trap techniques in *Drosophila melanogaster* to work on two related projects. For one project, a set of experiments has been worked out in advance to take the students from a behavior mutant (flightless), to a cloned and sequenced gene (gene for muscle myosin heavy chain protein), and an analysis of the gene. These experiments expose the students to a wide range of the common molecular techniques and demonstrate the logical progression of a research program. Techniques covered include: isolation of genomic and plasmid DNA, isolation of RNA, acrylamide and agarose gel electrophoresis, recombinant DNA techniques, characterization of mutants by Southern and Northern analysis, screening of a cDNA library, PCR, DNA sequencing and database analysis and protein isolation. The second project is an independent research project that starts with mutants of unknown genetic identity. The students use the techniques that they have learned during the first project to clone and sequence the gene and to begin to study the protein.

KEYWORDS: *Drosophila melanogaster*, project-based laboratory, molecular biology techniques

INTRODUCTION

The pervasive impact of modern molecular biology techniques on nearly all fields of biology and biomedicine make it imperative that students graduating from biology programs in our colleges and universities and entering careers ranging from basic research, biotechnology and medicine to K-12 teaching and science writing, have an understanding of these techniques and the concepts underlying them. To truly understand most techniques in molecular biology, students need not only a textbook explanation of the technology, but first-hand experience in the laboratory as well. There is a growing acceptance of the idea that students learn and retain best those concepts that they acquire through research or project-based learning (National Research Council, 1997; National Research Council/National Science Foundation, 1996; National Science Foundation, 1996). However, research-based learning is time consuming and does not seem compatible with the goal of covering a large amount of material in a short amount of time. We are faced, then, with two conflicting goals. The first is to introduce the students to a large number of modern molecular biology techniques; the second is to mimic the research setting and allow the students time for research-based learning. Whereas many colleges and universities attempt to provide this type of training through an independent research offering, a limited number of students can benefit from these experiences due to space and resource constraints. In addition, the range of techniques that the students are exposed to is often limited in these situations. The advanced molecular biology laboratory course described here, on the other hand, satisfies both of the above goals.

In this laboratory course, students work on two projects that run simultaneously. Both projects are based on P-element insertion mutations in *Drosophila melanogaster*. One project utilizes a mutation in a well-characterized gene. All of the results for this project are known to the instructor, all of the experiments have been pre-tested, and all of the biological products of the experiments have been stockpiled. In the second project, the students work in small groups on previously isolated mutants of unknown genetic identity. The first project runs smoothly and many different experiments can be done in a short amount of time. The second project gives the students the experience of carrying out their own research project.

Both projects start with the identification of mutant flies by behavioral testing. The mutant in the

first project has a recessive lethal, dominant flightless phenotype. The mutant phenotypes for the independent projects vary. The presence of the *lac Z* reporter gene in the P-element construct allows the students to quickly determine the gene expression pattern in both embryos and adults. The presence of a cloning vector in the P-element construct allows the students to recover a genomic clone of the DNA that immediately flanks the P-element insertion. In the process of obtaining this clone, the students learn a battery of standard recombinant DNA techniques. During the time that the students are learning and carrying out the procedures to obtain a genomic clone, they also complete a set of computer tutorials that provide information about the techniques used for the initial creation of P-element lines and for their subsequent genetic manipulation to create new mutants. Another tutorial demonstrates how one could genetically map the behavioral mutant by recombination and deficiency mapping. These tutorials not only provide background information on the P-elements and their use in *Drosophila* molecular genetics, they also demand that the student's apply the genetic concepts they learned in their prerequisite genetics lecture course.

When a genomic clone is obtained for the flightless mutant, the students are provided with sequence for the clone (which has been isolated in advance). The students then carry out an NCBI Blast analysis of the sequence. The *Drosophila* genome has been completely sequenced and is freely available on the Internet. The Blast analysis of the genomic clone identifies the mutated gene as the myosin heavy chain gene**.** The P-element insertion is 1 kilobase 5' of the first exon of the myosin heavy chain gene. The insertion does not disrupt the coding region for the gene but does interrupt the promoter region (Wassenberg et al., 1987).

The students next use their cloned DNA to carry out genomic Southern and Northern analyses which confirm the presence of a rearrangement in the region of the gene and a change in the transcription level. The genomic clone is also used as a probe in a cDNA library screen. The students isolate a cDNA clone of the gene and prepare the clone for expression by subcloning it into an expression vector. The students also isolate muscle proteins from wild type and mutant flies and use polyacrylamide gel electrophoresis to show the difference in the amount of myosin heavy chain protein. They use RT-PCR to demonstrate alternative splicing of exons in adult and embryonic forms of the transcript for myosin heavy chain.

As shown in Table 1, the schedule of laboratories for this course mimics the logical progression of a research project where the results of one experiment determine the next logical experiment and where the products of one procedure are the starting materials for the next procedure. The schedule of laboratories also simulates the normal operation of a research laboratory where many different experiments may be under way on the same day.

Table 1: *Advanced Molecular Biology Techniques: Schedule of Experiments*

Characterization of the Mutants	
Behavioral Testing	Laboratory 1
Creation of P-element Lines and Jump-Start Mutagenesis	Laboratory 1 Assignment
Genetic Mapping of the Mutants	Laboratory 2 Assignment
Gene Expression Pattern: *lacZ*	Laboratory 2 and 3
Plasmid Rescue	
Preparation of chromosomal DNA	Laboratory 3
Agarose gel of chromosomal DNA	Laboratory 4
Digestion of chromosomal DNA	Laboratory 4
Preparation of Competent cells	Laboratory 4
Ligation	Laboratory 4 Assignment
Transformation	Laboratory 5
Pick colonies and Start Liquid Culture	Laboratory 5 Assignment
Isolation of Plasmid DNA and test gel	Laboratory 6
Single and double digestion of plasmid DNA	Laboratory 6
Agarose gel of digested DNA	Laboratory 7
Analysis of sequencing data, database searches	Laboratory 7
Preparation of PRF of "unknown" for sequencing	Laboratory 8
Preparation of a frozen stock of new clones	Laboratory 8
Genomic Southern Analysis	
Preparation of chromosomal DNA	Laboratory 3
Digestion of chromosomal DNA	Laboratory 8
Gel of digested chromosomal DNA	Laboratory 9
Genomic Southern Blot	Laboratory 9
UV cross-link DNA to membrane	Laboratory 9 Assignment
Preparation of probe	Laboratory 12

Labeling of Probe	Laboratory 13
Pre-hybridization and hybridization of blot	Laboratory 13
Blot washes and Detection of Non-radioactive label	Laboratory 14
Analysis of results	Laboratory 15

Northern Analysis

Preparation of Total RNA	Laboratory 7 and 8
Preparation of Poly A^+ RNA	Laboratory 9
Northern Gel and Blot	Laboratory 10
UV cross-link RNA to membrane	Laboratory 10 Assignment
Preparation of probe	Laboratory 12
Labeling of Probe	Laboratory 13
Pre-hybridization and hybridization of blot	Laboratory 13
Blot washes and Detection of Non-radioactive label	Laboratory 14
Analysis of results	Laboratory 15

PCR Analysis of Splicing Variants

Preparation of Total RNA	Laboratory 7 and 8
Preparation of Poly A^+ RNA	Laboratory 9
Design Primers	Laboratory 10
RT-PCR	Laboratory 11
Test gel of PCR Product	Laboratory 12

Preparation and Labeling of DNA probe

Digestion of plasmid containing common gene coding sequence and agarose gel of digested DNA	*
Isolate PRF clone from gel (Geneclean™)	Laboratory 12
Check purity of Geneclean™ product by test gel	Laboratory 12
Label Geneclean™ product	Laboratory 13

cDNA Library Screen

Plate Library	Laboratory 11
Phage lifts and filter preparation	Laboratory 12
Preparation of probe	Laboratory 12
Labeling of Probe	Laboratory 13
Pre-hybridization and hybridization of filters	Laboratory 13
Filter washes and Detection of Non-radioactive label	Laboratory 14
Analysis of results	Laboratory 15

***in vivo* excision of cDNA**

Pick positive clones and elute	Laboratory 15
Grow host bacteria	Laboratory 15 Assignment
In vivo excision and plating	Laboratory 16
Plasmid preps of cDNA and test gel	Laboratory 17
Digestion of cDNA clones	Laboratory 17 Assignment
Test gel of digested cDNA clones	Laboratory 18

Subcloning of cDNA

Digestion of cDNA and vector for subcloning	Laboratory 18
Precipitation and Ligation reaction for subcloning	Laboratory 18 Assignment
Transformation	Laboratory 19
Plasmid DNA preparations of subcloned cDNA	Laboratory 20
Precipitate and run test gel	Laboratory 20
Digest subcloned cDNAs	Laboratory 20 Assignment
Gel of digested subclones	Laboratory 21
Overnight culture of clone with the insert in the correct direction for expression	Laboratory 21

Muscle Protein Preparation

Dissection and glycerination of flight muscle	Laboratory 21
Muscle protein preparation	Laboratory 23
Polyacrylamide gel electrophoresis	Laboratory 23

Expression of Cloned Gene

Start liquid culture for protein expression	Pre-laboratory 22 Assignment
Induce expression of protein	Laboratory 22
Purification of protein on Probond™ resin	Laboratory 22
SDS-PAGE gels (pre-cast0	Laboratory 23
Stain with Coomassie blue	Laboratory 23

* Prepared for students by technical assistant prior to laboratory when needed

In the second project, the students work in small groups (2-4) on different P-element insertion lines. All that is known about these lines is the cytogenetic map location and that the P-element insertion causes a recessive lethal mutation or some easily identified behavioral mutation. The schedule of experiments for this line begins in parallel with the experiments on the flightless mutant. The individual groups determine the reporter gene expression pattern for the affected gene and obtain a plasmid rescue clone. Since no previously stockpiled biological materials (i.e., results) are available when the students' results are less than optimal, the progress on these projects becomes asynchronized from the work on the flightless mutant relatively quickly. These projects afford the students the opportunity to test their understanding of the procedures and concepts that have been demonstrated using the flightless mutant. They also demand that the students learn to work in groups, to plan experiments, to trouble-shoot when the procedures do not give the expected products and to interpret their data and plan future experiments.

MATERIALS AND METHODS

Fly Stocks

Unless otherwise noted, all fly stocks were grown on standard cornmeal/agar medium at room temperature; no temperature-controlled incubators were available. All of the fly stocks used in this laboratory were obtained from the Bloomington Stock Center in Bloomington, Indiana. The P-element insertion line, harboring an insertion mutation in the myosin heavy chain gene, is BL# 10995[P {lacZ$^{P\backslash Z}$ w$^{+m/C}$ ampR ori = lacW}]. Many recessive lethal P-element lines are available at the Stock Center; these can be used as unknowns. Canton S and W1118 flies served as wild-type controls.

Background

All of the P-insertion lines used in this laboratory contain a p(lacW) construct insertion (Figure 1). The p(lacW) construct contains a *lac Z* reporter gene fused in frame to sequences in the second exon of the transposase gene from a native transposon. It also contains the mini-*white*$^{+}$ gene as a genetic marker and a pBR3322 vector for plasmid rescue (Bier et al., 1989). The laboratory exercises begin with computer tutorials that demonstrate how P-element insertion lines are created either by injection of embryos or by jump-start mutagenesis of established P-insertion lines. The computer tutorials can be found at (http://people.hofstra.edu/faculty/Beverly_Clendening/Adv_Molecular_Biology/index.html).

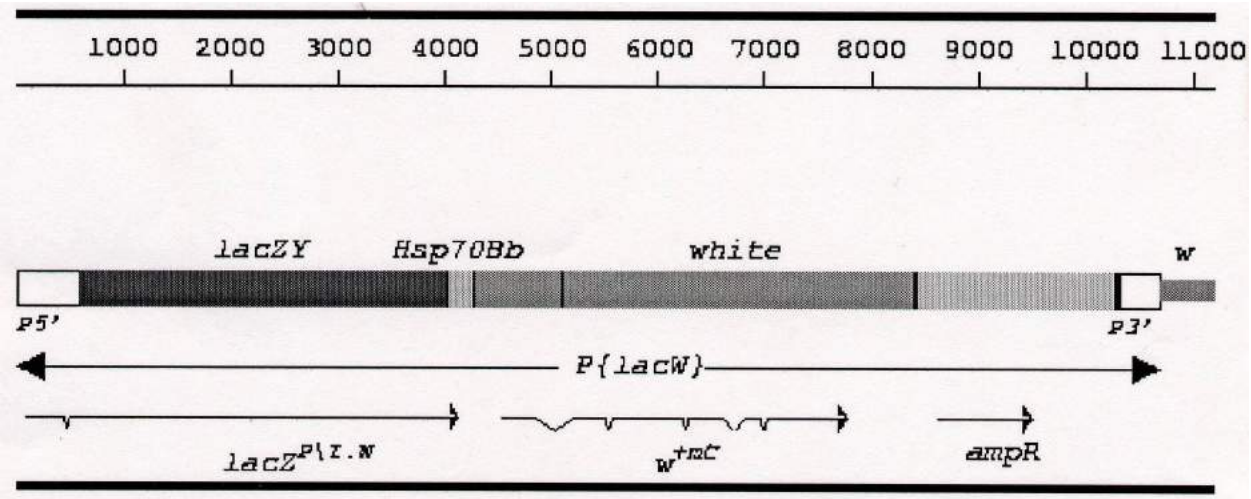

Figure 1. *Molecular map of the p(lacW) transposon showing the approximate placement of the lacZ gene, the promoter, the mini-white*$^{+}$ *gene and the modified pUC cloning vector. The scale at the top marks approximate kilobases.*

Characterization of the Mutant

Flight testing. The myosin heavy chain mutant has a recessive lethal, dominant flightless phenotype. These flies are maintained as heterozygotes over the *CyO* balancer chromosome. The balancer chromosome carries the dominant *Cy* gene; therefore, the flies have curly wings. Since curly-winged flies cannot fly, the 10995 line must be crossed into a wild type line to produce heterozygous flies with straight wings. The straight-winged myosin heavy chain mutants, carrying

one copy of the mutated chromosome, wild-type flies and randomly selected P-element line flies were flight-tested in a protocol adapted from (Drummond et al, 1991). Briefly, flies were released from vials in a Plexiglas flight-testing chamber (Figure 2) with a light source at the top of the chamber; this was the only light source in the room. As the flies left the vial they did one of several things. Some flies immediately flew vertically or diagonally upward toward the light source. These were given a score of 3 and were considered normal. Some flew horizontally; these were given a score of 2. Some flies flew in a downward direction and often had to be coaxed to attempt flight by gentle shaking of the vial. These were given a score of 1. Finally, some flies fell straight down; these always had to be coaxed to attempt flight. These flies were given a score of 0. The students generated a weighted score for each line of flies tested. The flies were not anaesthetized prior to flight-testing. (If flies are anaesthetized at some point prior to flight testing, they should be allowed two days to recover before flight-testing. Flies should also be examined in their vials for wing and other anatomical defects prior to testing.)

Figure 2. *Flight Testing. Flies are released inside a Plexiglas box. The room is darkened and a light source is placed at the top of the testing chamber. Normal flies will fly toward the light source.*

Adult and embryonic *lac Z* expression pattern

Adult. The students were provided with longitudinal and transverse cryostat sections (10 µm) of fresh frozen 1-2 day-old flies from line 10995 and other P-element lines. They fixed the sections in 2% paraformaldehyde in 1X PBS for 15-20 minutes, washed the slides with 1X PBS and incubated them overnight at 37˚C in staining solution (5 mM ferricynaide, 5 mM ferrocynanide in 1X PBS plus 0.2% (w/v) X-gal dissolved in DMSO). Incubation took place in a humidified chamber consisting of a plastic container lined with damp paper towels. After staining, the sections were washed with 1X PBS, mounted in 10% glycerol and examined under brightfield optics.

Embryos. Flies from line 10995 and other P-element lines were allowed to lay eggs on grape agar plates for 4-24 hours. Embryos were collected and dechorionated by soaking in 50% bleach for 15 minutes. After washing 2-3 times with distilled water, the embryos were fixed for 2 hours in 20% n-heptane saturated with 8% paraformaldehyde. Fixed embryos were washed with PBST (1X PBS, 1% Triton X-100) and incubated for 1-5 days in with staining solution (5 mM ferricyanide, 5 mM ferrocynanide in PBS plus 0.2% (w/v) X-gal dissolved in DMSO) at 37˚C. Staining was followed by washes with 1X PBS. Whole embryos were mounted in 10% glycerol and examined under brightfield optics.

Deficiency Mapping of the Mutant

A computer-based tutorial that allows the students to deficiency map the mutation in line 10995 is available at the course website (http://people.hofstra.edu/faculty/Beverly_Clendening/Adv_Molecular_Biology/index.html). In this tutorial the students are first introduced to the theory underlying deficiency mapping. Then a set of deficiency lines covering the second chromosome is displayed. A button click on each deficiency line displays a sample phenotype distribution of the F1 generation when the deficiency line is crossed with the 10995 mutant. From these manipulations, the students uncover deficiency lines that do not complement the mutation in line 10995. Next, the students are instructed to use these results to determine the deficiency overlap between the non-complementing deficiency lines. This information is obtained from *Flybase*, a *Drosophila* genome database (http://flybase.bio.indiana.edu:82). The students must locate the reference to each deficiency line and determine what cytological map area is missing. When they map these deficiencies, the students discover that a small area of chromosome 2 is common to all of the non-complementing lines. The mutation in line 10995 is contained in this area of overlap. The students are instructed to use the "Cytosearch" function of *Flybase* to find all of the possible genes, both known genes and candidate genes, for P-insertion line 10995.

General molecular techniques. Standard protocols (Sambrook and Russell, 2001) for agarose gel electrophoresis, use of restriction and modifying enzymes, preparation of *Drosophila* genomic DNA, small scale plasmid DNA preparation, RNA preparation and screening the cDNA library were used throughout the procedures.

Preparation of radioactive and non-radioactive probes

Plasmid rescue clones (see below) were digested with *SacII* and *BamHI* to separate the vector from the insert DNA. The fragments were size fractionated in an

agarose gel. Because BamHI cuts internally in the plasmid rescue clone, several fragments are obtained in addition to the 1.7 kb vector. These fragments were excised from the gel and purified using a GeneClean™ kit (Obiogene). The plasmid rescue clone can also be digested with *SacII* and *XbaI*, leaving the ~21Kb plasmid rescue clone intact. However, this large fragment is very difficult to purify without shearing.

Labeling DNA

Two methods were used to label the plasmid rescue DNA. Students used photobiotin (Sigma) labeling. Photobiotin was mixed with DNA in a 3:1 ratio by concentration. The mixture was placed under a 275-watt heat lamp for 15 minutes and then extracted with butanol to remove unincorporated biotin. A Rediprime™ Random Prime Labeling kit (Amersham) was used to make ^{32}P-labeled probes. Students did not handle the ^{32}P-labeled probe.

Hybridization Procedures

Blots and filters were pre-hybridized and hybridized in a solution containing 5X Denhardt's solution, 5X SSC and 0.5% SDS. Pre-hybridization solution also contained 1.0 mg/ml denatured salmon sperm DNA. Pre-hybridization was carried out at 65˚C for 1-2 hours. Labeled, denatured probe was added to the hybridization solution at 1 ng/ml. Hybridization was carried out at 65˚C overnight. After hybridization the blots and filter were washed twice in 2X SSC, 0.5% SDS at 65˚C for 20 minutes and once in 0.2X SSC, 0.5% SDS at 65˚C for 20 minutes.

Plasmid Rescue. Established protocols (Bier et al., 1989) were used to obtain a fragment of DNA flanking the P-element insertion in line 10995 flies. Briefly, 1-3 days old 10995 line flies were homogenized in buffer containing 100mM Tris-HCl, pH 9.1, 100mM NaCl, 200mM sucrose, 50 mm EDTA and 0.5% SDS. The homogenate was treated with RNase (50µg/ml) and Proteinase K (50µg/ml) and purified by phenol/chloroform extraction. 5ug of the isolated chromosomal DNA was digested overnight at 37˚C with *SacII* (Promega). The digested fragments were ligated overnight at 16˚ C with T4 DNA ligase (Promega) under conditions which discourage intermolecular reactions (large volume). The ligated DNA was used to transform XL-1 Blue cells (Stratagene) which had been made competent for electroporation following the protocol provided by Epppendorf. Electroporation was carried out at 1.5kV in a 1 mm gap cuvette. Cells were allowed to recover in SOC medium (0.5% yeast extract, 2% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$ 20 mM $MgSO_4$, 20 mM glucose) for 1-2 hours at 37˚C and were then plated on LB agar plates with ampicillin and tetracycline. Colonies with both tetracycline resistance, conferred by the XL1-Blue cells, and ampicillin resistance conferred by the P-element cloning vector are assumed to carry a plasmid containing the vector portion of the P-element plus a fragment of the DNA flanking the P-element. Colonies were grown overnight in LB plus ampicillin and tetracycline (75µg/ml each) and the plasmid DNA was recovered from the bacterial cells using a standard alkaline lysis plasmid preparation protocol. The plasmid DNA was digested with both *SacII* and *BamHI* or *SacII* and *XbaI* to separate the cloning vector from the flanking *Drosophila* DNA. The digested DNA was size fractionated to demonstrate that the expected fragments were present. Because *BamHI* cuts internally in the plasmid rescue clone, several fragments were obtained in addition to the 1.7 kb vector. The plasmid rescue DNA was prepared for sequencing using either the Qiagen Plasmid Mini-Prep Kit or Promega SV Wizard DNA Preparations. Other preparations can be used based on the requirements of local sequencing facilities. Since the p(lacW) construct does not contain the standard forward and reverse sequencing primer binding sites, the students used the P-element sequence, which can be found in *Flybase*, and either the *MacVector* or *Jellyfish* DNA analysis programs to pick sequencing primers. The primer I used for sequencing is shown in below. The sequence obtained from this primer contains 53 base pairs of the 5' end of the P(lacW) construct followed by *Drosophila* sequence at the insertion site.

5' – AAGTGGATGTCTCTTGCCGACG – 3'

I do not actually sequence the plasmid rescue fragment each time a new class isolates it; I give the students the sequence I have obtained previously.

Sequence Analysis. The NCBI and BDGP databases were used to analyze the plasmid rescue sequence.

Northern Analysis. To obtain total cellular RNA, whole adult *Drosophila* were homogenized and allowed to incubate at 4˚C overnight in a 6M urea/3M LiCl homogenization solution. The suspension was then pelleted, resuspended in a solution containing 10 mM Tris HCl, pH 7.5, 10 mM EDTA and 1% SDS, and purified by phenol:chloroform extraction. Poly A^+ RNA was recovered from total RNA by the use of an oligo dT cellulose (Sigma) column. RNA was fractionated on 1.5% agarose/17% formaldehyde gels and blotted overnight onto Nytran Plus™ transfer membrane. Each group of students prepared two identical blots, one was used with a ^{32}P-labeled probe, the other was used with biotin-labeled probe. The blots were UV cross-linked and probed with labeled plasmid rescue fragments, following the pre-hybridization and hybridization protocols outlined above. Blots containing ^{32}P-labeled probe were placed on Kodak BioMax film and the film was developed after 1-2 days. Blots containing biotin-labeled probe were washed in blocking solution (1.0 M NaCl, 1 M Tris-HCl, pH 7.5, 2.0 mM $MgCl_2$, 0.05% Triton -X 100, 3% bovine serum albumin) for 30 minutes shaking gently

to prevent non-specific binding of the strepavidin alkaline phosphatase (SAP) conjugate to the nitrocellulose and nylon. The blots were then incubated in 10 ml Buffer A (1.0 M NaCl, 1 M Tris-HCl, pH 7.5, 2.0 mM $MgCl_2$, 0.05% Triton -X 100) containing 25 µl of strepavidin-alkaline phosphatase conjugate) for 25 minutes with gentle shaking. After incubation the blots were washed 3X for 10 minutes each with 50 ml of Buffer A and for 5 minutes with Buffer C (0.1M NaCl, 0.1M Tris-HCl, pH 7.5, 10 mM $MgCl_2$). Finally, the blots were incubated in dye solution (64 µl of NBT (50 mg/ml)/32 µl BCIP (5-bromo-4-chloro-3-indolyl phosphate) in 10 ml Buffer C under reduced light for 30 minutes to 3 hours. When a blue-black reaction product was seen, the reactions were stopped by washing the blot with 1.0 mM EDTA and dried. After drying the blots should be stored away from strong light.

Southern Analysis. 10 µg of chromosomal DNA from wild type and mutant flies was digested overnight with *SacII* and fractionated on a 0.7% agarose gel. The fractionated DNA was depurinated by soaking in 0.25 M HCl for 10 minutes and then denatured in 1 M NaCl, 0.5 M NaOH. This was followed by neutralization in 1 M NaCl, 0.5 M Tris-HCl, pH 8. The DNA was blotted overnight onto Nytran Plus™ transfer membrane. Each group of students prepared two identical blots, one was used with ^{32}P-labeled probe; the other was used with the biotin-labeled probe. The DNA was UV cross-linked to the membranes and probed with labeled plasmid rescue fragments following the pre-hybridization and hybridization and detection protocols outlined above.

cDNA Library Screening. The fragments recovered by plasmid rescue were used as a probe to screen a λ ZAP™ adult *Drosophila* cDNA library (provided by Stratagene, Inc.) at high stringency using the protocols provided by Stratagene. Since time was available during the laboratory for only one round of isolation, the library was "spiked" with a cDNA clone of the myosin heavy chain gene that had been obtained previously. The phage were added to host bacteria (XLI-Blue) at five dilutions (10^{-1} - 10^{-6}). Plates giving well-defined, isolated plaques were used for plaque lifts. Plaques were lifted onto nitrocellulose filters using standard protocols (Sambrook and Russell, 2001). Phage DNA was UV-crosslinked onto the filters and the filters were probed using the prehybridization and hybridization procedures outlined above. The "spiking" procedure insured that all students would detect positive clones that could be isolated after one round of screening.

In vivo Excision of Plasmid cDNA. The λ ZAP vector is designed to allow the in vivo excision and recircularization of cloned insert to form a circular molecule containing the phagemid, Bluescript SK (-) and the cloned insert. A detailed description of the in vivo excision process is available at http://www.stratagene.com/vectors/cloning/zap2.htm. To accomplish the in vivo excision, positive plaques were eluted in SM buffer (100µM NaCl, 1mM $MgSO_4$•$7H_2O$, 20mM Tris-HCl (pH7.5), 0.01% gelatin) plus 0.2% chloroform for four hours with gentle agitation. 250 µl of this phage stock plus ExAssist™ helper phage (> 10^6 pfu) were used to infect 200µl of XL1-Blue host cells. During this incubation period the phagemid is secreted from *E-coli*. After a 2-2.5 hour incubation period the mixture was heated to 75˚C for 20 minutes to kill the bacteria. The filamentous phage particles were recovered by centrifugation. These phage particles were used to infect SOLR host bacteria. Infected host cells were grown on LB plates supplemented with ampicillin (LB-amp plates). Colonies were grown in LB-amp liquid cultures and the plasmid DNA was extracted following a standard alkaline lysis plasmid DNA preparation protocol (Sambrook and Russell, 2001). The plasmid DNA was digested with EcoRI to isolate the cDNA insert from the pSK vector.

Subcloning the cDNA into an Expression Vector. The expression vector that was used for the class was pTrcHis2 (Invitrogen). This vector provides high level regulated transcription from the trc promoter and the lacO operator and $lacI^q$ repressor gene for transcriptional regulation of any *E. coli* strain. It also has a C terminal polyhistidine tag for purification and detection. Three vectors are available; each has the C terminal tag coding sequence in a different reading frame relative to the multiple cloning sites to simplify in-framing cloning. Many other commercial vectors would work as well. The cDNAs and vector were digested with EcoRI. The Mhc transcript has two internal EcoRI sites, one before the start codon and the other 5bp from the stop codon. The cDNA can be digested completely since loss of the end fragments is inconsequential to the expression of the gene from the expression vector. After digestion the cDNA clone was ~ 5 kb in length. The expression vector has an EcoRI site in the multiple cloning region. Use of the "A" version of the pTrcHis2 vector allows the EcoRI digested cDNA to be inserted in the vector in the correct reading frame for expression. The cDNA clone and the expression vector were digested overnight, precipitated to remove enzyme and salts and ligated overnight in an insert:expression vector molecule ratio of 3:1. The ligated DNA was used to transform One Shot® cells by heat shock following the instructions provided by Invitrogen. This cloning system uses blue/white selection so bacteria that incorporated plasmid with vector plus inserted DNA appear white on plates treated with X-gal (5-bromo-4-chloro-3-indolyl β-D-galactopyranoside) and IPTG (isopropyl β-D-thiogalactopyranoside). Bacteria that incorporated only the ligated vector will turn blue. White colonies were grown overnight, plasmid DNA was isolated and a sample of the plasmid DNA was run on an agarose

gel. Since pTrcHis2 is a non-directional cloning vector, plasmids of the expected correct size (~9 kb, ~5 kb insert in the 4 kb vector) were digested with XbaI and NarI to determine the orientation of the insert in the vector. There is a recognition sequence for XbaI in the multiple cloning site of the expression vector downstream of the EcoRI. insertion site. The Mhc sequence contains no XbaI recognition site. There is a recognition sequence for Nar I, 3.5 kb from the 5' end of the Mhc fragment. There are no recognition sequences for Nar I in the vector. A diagnostic gel was run to reveal the orientation of each cloned cDNA. If the cDNA was cloned into the expression vector in the correct orientation for expression, the Xba I/Nar I digest should yield 1.5 and 7.5 kb (approximate) fragments. If the orientation is reversed 3.5 and 5.5 kb (approximate) fragments will be obtained.

Expression and Purification of the Subcloned cDNA. Bacteria containing subcloned cDNA in the correct orientation for cloning were streaked on fresh LB-amp plates and single colonies were grown overnight at 37˚C in SOB broth. The next day, 1 ml of the overnight culture was used to inoculate 50 ml of SOB and the culture was grown to an OD_{600} = 0.6. IPTG was then added to the culture to a final concentration of 1 mM and the cells were allowed to grow for an additional 3 hours. The cells were harvested by centrifugation (3000 x g for 10 minutes at 4 ˚C). The harvested cells were resuspended in a buffer containing 20mM $NaPO_4$, 500 mM NaCl, pH 7.8. Lysozyme (100 µg/ml) was added and the cell suspension was incubated on ice for 15 minutes. The suspension was sonicated with 2-3 10-second bursts at medium intensity while holding in ice. Lysis was completed by three freeze-thaw cycles (liquid nitrogen/37˚C water bath). RNase and DNase were added to a final concentration of 5 µg/ml each and the preparation was incubated at 37˚C for 15 minutes. Insoluble debris was removed by centrifugation (3000 x g for 15 minutes). The lysate was cleared by passage through a 0.8 µm syringe filter.

The ProBond™ Purification System (Invitrogen) was used to purify the polyhistidine-tagged expressed protein following the instructions provided by the manufacturer. The size and estimated concentration of the purified product were checked by fractionation in a pre-cast 12% Tris-glycine polyacrylamide gel.

Isolation of Muscle Proteins. Indirect flight muscles were dissected from the thoraces of 5 adults from a W1118 line and of 5 adults from line 10995. The tissue was homogenized lightly, and placed in York Modified Glycerol Solution (20 mM Na-phosphate buffer, pH 7.0, 50% glycerol, 0.5% Triton X-100, 2 mM $MgCl_2$, 1 mM NaN_3, 1 mM DTT) at -20˚C for two days. The tissue was pelleted by centrifugation and the supernatant was removed. The tissue was resuspended in Rigor Buffer (10 mM Na-phosphate buffer, pH 7.0, 100 mM NaCl, 2 mM $MgCl_2$, 2 mM EGTA, 0.1 mg/ml soybean trypsin inhibitor, 1 mM DTT) with Triton X-100 and homogenized lightly. This was repeated three times. The tissue was then rinsed 2 X in Rigor Buffer without Triton X-100. Finally the buffer was removed and the tissue was re-suspended in Sample Buffer (50 µM Tris-HCl, 10% glycerol, 0.5%SDS, 0.5%beta-mercaptoethanol, 0.5% bromophenol blue). The sample was heated at 95˚C for 5 minutes and the entire sample was loaded into one well of a pre-cast 12% Tris-glycine polyacrylamide gel. Gels were stained with Coomassie stain (50% methanol, 0.05% Coomassie brilliant blue R-250, 10% acetic acid) for 2-4 hours and then destained with 3-4 washes of destaining solution (7% acetic acid, 5% methanol) over the next 12-24 hours.

RT-PCR Analysis of Alternative Slicing. The students used previously isolated Poly A^+ RNA from embryonic and adult wild type flies to obtain PCR products of the 3' end of the *Mhc* transcript. Several alternate *Mhc* transcripts are generated in *Drosophila* by the selective use of alternative polyadenylation sites and by the alternative splicing of exons. Exon 18 is not included in the embryonic and some isoforms of the adult transcript, but is included in other isoforms of the adult message (Rozek and Davidson, 1986). Therefore, when oligo dT is used as the primer for reverse transcription and sequences from exons 17 and 18 are used as primers for PCR, a product is obtained from the adult cDNA sample but no product is obtained from the embryonic cDNA sample. Students used the documented sequence of the *Drosophila Mhc* gene to pick primers. The primers used by the class are given below.

Forward primer starts on plus strand within exon 17:

5'-CTGGACGAACTCCTGAACGAAG - 3'

Reverse Primer starts on minus strand within exon 18:

5'-CCATTGATTTTGATTGGGGTGGC-3'

Product size: 814 nucleotides

The Accutaq™ RT-PCR kit (Sigma) was used to generate the RT-PCR products. 1µg of $polyA^+$ RNA from 2-3 day old adults and 1µg of $polyA^+$ from 12-24 hour embryos were used in separate reactions as the template for reverse transcription with the oligo dT primer. The resulting cDNA products were amplified using the primers given above. As an internal control for cDNA quality, β actin standard primers (Ambion) were also used to amplify a product from each cDNA pool. Samples of the four PCR products were run on a standard 0.8% agarose gel.

Table 2 lists equipment and general and molecular biology supplies required for the course.

***Table 2**. Equipment and Supplies required for the Laboratory*

EQUIPMENT	GENERAL CHEMICAL SUPPLIES
3 Benchtop microcentrifuges	ampicillin
microwave oven	tetracycline
3 electrophoresis set-ups	agar
3 constant voltage power supplies	acetic acid - glacial
cryostat	n-heptane
2 vertical gel apparatus	2-butanol
3 vortex mixers	embedding medium for cryostst
2 hotplate/stirrer	Triton X-100
dry bath incubator (6 block)	glycerol
2 general purpose water baths	dimethylsulfoxide
dual range analytical balance	EDTA
pH meter	ingredients for L-Broth, NZYand SOC media
refigerator with non-defrosting freezer	phosphate-buffered saline
platform incubator/shaker	various salts, acids and bases
refrigerated microcentrifuge	fly food ingredients
UV crosslinker	SDS
electroporator	paraformaldehyde
photodocumentation system	ethanol
thermal cycler- 48 wells	isopropanol
autoradiography cassettes and enhancer screens	
10 sets of pipettors (each set with 3 pipettors, 1-2µl, 20-200 µl, 200-1000µl)	**MOLECULAR BIOLOGY CHEMICAL SUPPLIES**
3 desk-top computers with internet and printers	lysozyme
MacVector Program	restriction endonucleases
infrared lamp	T4 ligase
visible light view box	DNA labeling kit
NON-CHEMICAL SUPPLIES	polyethylene glycol 400
microcentrifuge tubes	PCR supplies
microtube storage racks and rack holders	reverse transcription supplies
conical tubes	primers
conical tube storage racks	proteinase K
homogenizers	RNase A
glass slides and cover glass	Pre-cast acryamide gels
test tube storage boxes	I kb laddder
autoradiographic film	RNA ladder
blotting paper	diethylpyrocarbonate
nylon/nitrocellulose transfer membranes	ethidium bromide
culture plates	Denhardt's solution
microcentrofuge tubes	agarose
pipette tips	loading buffer
spin columns for RNA	phenol
electroporation cuvettes	chloroform
ice buckets	MOPS buffer
disposable transfer pipets	oligo-dT cellulose
fly vials and storage containers	photobiotin
STANDARD EQUIPMENT *	nitro-blue tetrazolium
chemical fume hood	5-bromo-4-chloro-3-indolyl phosphate
high speed centrifuge	Strepavidin alkaline phosphatase
autoclave	XL1-Blue cells
microscopes	Plasmid DNA Purification kit
37˚C incubator	cDNA library
water purification system	Gene Clean kit
RESUABLE SUPPLIES*	Protein purification kit
glassware: beakers, flasks, bottles, etc.	Expression vector
Oakridge tubes	X-gal
carboys	IPTG
test tube racks	

* Standard equipment and reusable supplies expected to be available in all Biology Departments

Online Manual

Detailed protocols that serve as the laboratory materials for the class are available online at (http://people.hofstra.edu/faculty/Beverly_Clendening/Adv_Molecular_Biology/index.html)

RESULTS

Tutorials: Creation of P-Element Lines and Jump-Start/Jump-Out Mutagenesis

The tutorials were useful not only as introductory material but also as a review of basic concepts in classic genetics. They are interactive in that, at many points in the program, the students must answer questions before proceeding. The questions check the students' understanding of basic genetic concepts such as independent segregation. The questions that can be answered as a part of a multiple-choice format require that the correct answer be provided before the student can continue. Other questions require the students to give a more extensive answer. The answers are typed into the appropriate box. The student cannot continue until something is entered into the box, however, the program contains no mechanisms for checking the answers. I require that the students submit their answers to these questions to me.

Characterization of the Mutant

Flight testing. Heterozygous flies from line 10995 are not able to fly and invariably obtained a score of 0 in flight-testing. The results from this behavior test are unambiguous. Flies in some of the "unknown" P-insertion lines also do not fly perfectly or at all even when the balancer chromosome is replaced by a wild type chromosome. Mutations in many different genes can cause flight impairment. The results of this test in combination with the results from the *lacZ* staining procedure provide an opportunity to impress on the students the need for critical evaluation of results and the need to consider different types of data in the characterization of a mutant.

lac Z reporter gene expression

Diffuse staining of mesodermal tissue was seen in X-gal treated embryos from line 10995 starting at Stage 10 (Figure 3A). *lac Z* reporter gene expression was prominent in all line 10995 adult somatic muscle tissue (Figure 3B and 3C). *lac Z* expression was not seen in any other adult tissue.

Deficiency Map. P-element insertion line 10995 and all of the second chromosome deficiency lines presented in the web-based tutorial are balanced over the *Cy*O balancer chromosome that carries the dominant curly wing mutation. Flies in the 10995 line and all of the deficiency lines, therefore, will have curly wings. Flies that carry one copy of the 10995 mutation and one copy of the deficiencies will have no balancer chromosome and will have straight wings. Therefore, when crosses between line 10995 flies and deficiency line flies result in no straight-winged progeny, the parent lines do not complement and the mutations in the two lines are likely to affect the same gene. The following deficiencies fail to complement line 10995:

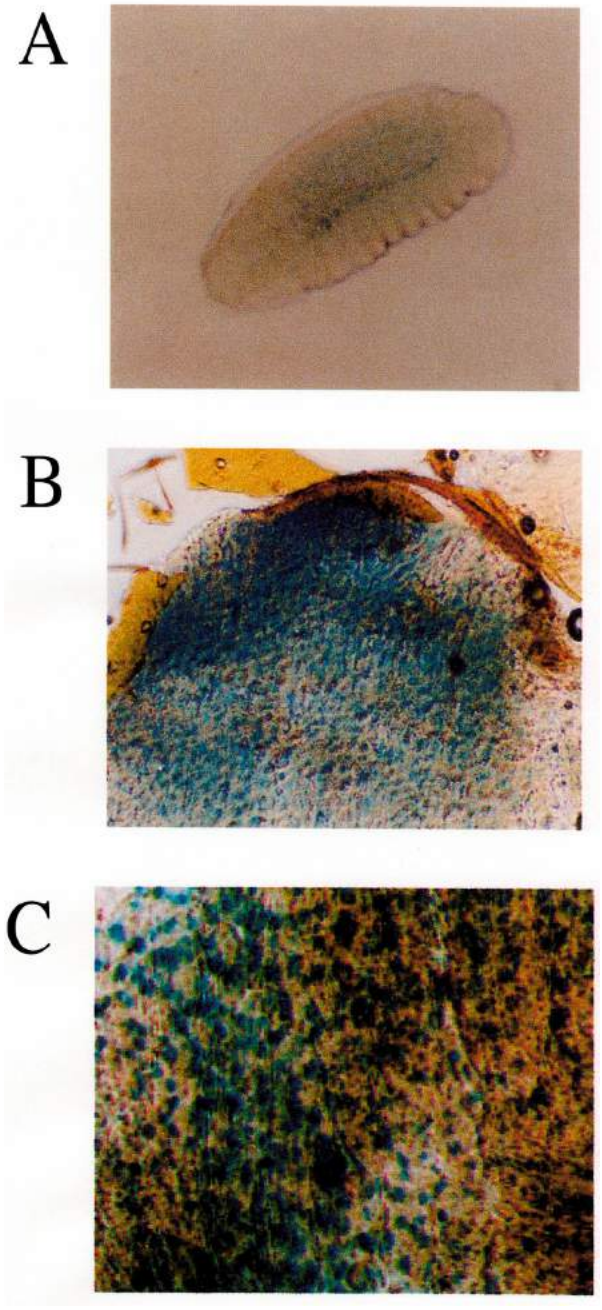

Figure 3. *lac Z Expression Pattern. A). Photomicrograph of a whole mount of a late stage embryo (200X). The lac Z reporter gene is expressed diffusely in mesodermal tissue. B) Photomicrograph of an X-gal treated sagittal cryostat section through the head and thorax of an adult Drosophila showing the flight and jump muscles (200X).C) Photomicrograph of an X-gal treated sagittal section through the thorax of an adult Drosophila showing the stained flight muscles (400X). This is the same section as seen in B.*

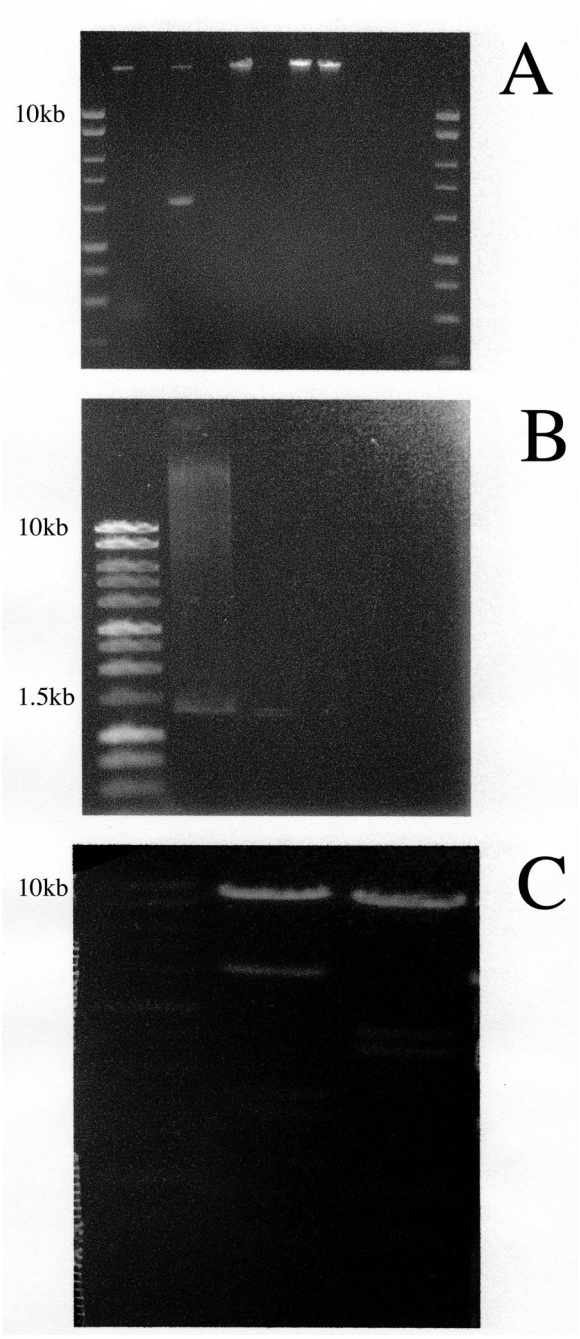

Figure 4*. Plasmid rescue clone from line 10995. A) Agarose gel of plasmid rescue clone DNA. Different lanes are samples from individual students. The plasmid rescue clone runs well above the 10 kb step of the ladder. B) Agarose gel of SacII and XbaI digest showing the 1.7 kb vector and an ~ 21 kb plasmid rescue fragment. Because of its large size, it is difficult to obtain intact plasmid rescue fragment. C) Agarose gel of SacII and BamHI (lane 2) and SacII and Pst I (lane 3) digests of the plasmid rescue clone. Because BamHI and Pst I cut internally in the plasmid rescue fragment, several bands, in addition to the 1.7 kb band representing the isolated vector, can be seen.*

Non-complementary Deficiency Lines

1) Df (2L)cact-255rv64	breakpoints: 35 F6-12, 36D
2) Df (2L)TE35D16	breakpoints: 35C1, 36A1-9
3) Df (2L)TE35D-23	breakpoints: 35B4, 36A8-9
4) Df (2L)H20	breakpoints: 36A8-9, 36F 1
5) Df(2L)3180	breakpoints: 36A8-9, 36E1-2
6) Df (2L)42385	breakpoints: 36A8-9, 36E3-4

A map showing one student's determination of the overlap between these deficiencies is shown in the *Sample Results* section of the Course Web site. The cytological map location that is common to all of the non-complementary lines is 36A8-9. A cytological search of this area in *Flybase* uncovers several possible mutant genes. Among these are *Cyt-b5-r, Mhc, Ifm(2)RU2* and *Ifm(2)RU1*. All of these are genes that have mutant phenotypes that make them candidates for the gene disrupted in line 10995. In particular, some *Mhc* alleles and the *IFM* mutants have recessive lethal, dominant flightless mutant phenotypes. The *IFM* alleles are mutant alleles of *Mhc.*

Plasmid Rescue and Analysis of Plasmid Rescue Fragment Sequence. A 21 kb *SacII* plasmid rescue fragment was obtained. A gel of the plasmid preparations of the rescue clones obtained by some of the class participants is shown in Figure 4A. Digestion with both *SacII* and *BamHI* or with *SacII and Pst I* followed by size fractionation by agarose gel electrophoresis reveals the separation of the vector from the flanking *Drosophila* DNA, *BamH and Pst I* cut several times internally in the plasmid rescue fragment (Figure 4C). The entire plasmid rescue fragment can be separated from the vector by a *SacII* and *XbaI* digestion but the large clone is prone to degradation as shown in Figure 4B. The plasmid DNA was sequenced (San Diego State University DNA Microchemical Core Facility) using an ABI Prism 377 DNA Sequencer. Sequence obtained using primers from the 3' end of the plasmid rescue clone is shown below.

Plasmid Rescue Fragment Sequence – Line 10995

```
AAGTGGATGTCTCTTGCCGACGGGACCACCTTAT
GTTATTTCATCATGGGCGAATTACTGGCGAAATG
ATTTCATACACAAATACCTGTGTGGCCGAGACAT
ATGCGTATGCATACTATAGAAAATAGATTTAGAA
TACTCGAATTCGTTGTCGGCTCATATACATGGGC
GAAATAATTTCGAATATGTTTTAAAAATAACCAA
AGACATTAGAAAGAGATCGCCAATACTTATACAT
TATGTCTATGTGTGCCATGTGGTAGCATGAGCCA
AAAAGCTTCTCGAAATTACGAATTACATATAGAC
GAATATGTATGTGACTTTAGTTTCGAAATAATTT
CGAGAATTTTAAAAATAACGCATTCGTTAAAAGT
TCGCGTCAATTCGAATCGAATTTTCGATTGTCGA
TTTAGTGTGGATTGTCGAAAATCGTTCCGCCTTC
GAAGTTTACTGAAAGGAATCATTGCGATCTCGTG
AATTGCTTGTATGAGAACACGCCACCATATCGAG
```

ATACCTGTTCAAAATATCGAAATCGCGTTCCAAA
CGGTAGTAGTTACCAGTTGAGTGAGTTGTGGTGA
CAGTTTAACCTCTCTACATTTGTAATAATAAGCA
TTGTTTTGGCGTATCAATAGTCTGTAGATTTTTT
CACAAAGAAACCTGGAGAAATGTACACAAATATC
TAAATGAAAATTGGCGTTACGTAACAAATACCAA
CGCCCCGAAAATCCGTATATATGCAAAGCATTCT
GATATATATATATATAAGTGGTATACATATACAT
ATTCGTATCGGATCCGTGATCTATATTTAGGCGA
CAACCCACACAATTCACTTGTTGTCTGTTTATAA
CAAAAACAACAACACAA

Sequence Analysis

The P-element insertion in line 10995 is in the 5' untranslated region of *Mhc* as shown by NCBI blast analysis of the plasmid rescue clone sequence (Figure 5).

```
>gb|J02188.1|DMMYHB D.melanogaster myosin heavy chain gene, 5' end
          Length = 4676

 Score = 1122 bits (566), Expect = 0.0
 Identities = 683/709 (96%), Gaps = 16/709 (2%)
 Strand = Plus / Plus


Query: 51   cgaattactggcgaaatgatttcatacacaaaatacctgtgtggccgagacatatg--cgt 108
            ||||||||||||||||||||||||||||||||||||||||||| |||| ||||||   |||
Sbjct: 1143 cgaattactggcgaaatgatttcatacacaaaatacctgtgtgaccgaaacatatgtgcgt 1202

Query: 109  atgcatactatagaaaatagatttagaatactcgaattcgttg-tcgg-ctcatatacat 166
            |||  ||||||||||||||||||||||||||||||||||||| |||| | |||||| ||
Sbjct: 1203 atgg-tactatagaaaatagatttagaatactcgaattcgttgatcggacacatatatat 1261

Query: 167  gggcgaaataatttcgaatatgttttaaaaataaccaaagacattagaaagagatcgcca 226
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1262 gggcgaaataatttcgaatatgttttaaaaataaccaaagacattagaaagagatcgcca 1321

Query: 227  atacttatacattatgtctatgtgtgccatgtggtagcatgagccaaaaaagcttctcgaa 286
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1322 atacttatacattatgtctatgtgtgccatgtggtagcatgagccaaaaaagcttctcgaa 1381

Query: 287  attacgaattacatatagacgaatatgtatgtgacttagtttcgaaataatttcgagaa 346
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1382 attacgaattacatatagacgaatatgtatgtgacttagtttcgaaataatttcgagaa 1441

Query: 347  ttttaaaaataacgcattcgttaaaagttcgcgtcaattcgaatcgaattttcgattgtc 406
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1442 ttttaaaaataacgcattcgttaaaagttcgcgtcaattcgaatcgaattttcgattgtc 1501

Query: 407  gatttagtgt-ggattgtcgaaaatcgttccgccttcgaagtttactgaaaggaatcatt 465
            |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1502 gatttagtgtgggattgtcgaaaatcgttccgccttcgaagtttactgaaaggaatcatt 1561

Query: 466  gcgatctcgtgaattgcttgtatgagaacacgccaaca-tatcgagataccgttcaaaa 524
            |||||||||| |||||||||||||||||||||||||| |||||||||||||| ||||
Sbjct: 1562 gcgatctcgtaaattgcttgtatgagaacacgccaccattatcgagataccgtttaaaa 1621

Query: 525  tatcgaaatcgcgttccaaacggtagtagttaccagttgagtgagttgtggtgaca---g 581
            |||||||||| ||||||| ||||||||||||||||||||||||||||||||||    |
Sbjct: 1622 tatcgaaat-gcgttcc-aacggtagtagttaccagttgagtgagttgtggtgaccagttg 1679

Query: 582  tttaacctctctacatttgtaataataagcattgttttggcgtatcaatagtctgtaga- 640
            |||||||||||||||||   |||||||||||||||||||||||||||||||||||||
Sbjct: 1680 tttaacctctctacatttg---taataagcattgttttggcgtatcaatagtctgtagat 1736
```

Figure 5. *Results of the Blast Analysis of sequence from the plasmid rescue clone showing the alignment between the sequence to be analyzed (query) and the nearly identical sequence found in the database (sbjct).*

Genomic Southern Analysis

Genomic southern analysis was used to verify that the plasmid rescue clone that was isolated is actually the DNA that lies adjacent to the P-element insertion. Genomic DNA from wild type and mutant flies was digested with *SacII*. *SacII* cuts at the 3' end of the vector within the P-element, at a site greater than 21 Kb downstream from the 3' end of the P-element insertion and at a site 4 kb upstream from the 5' end of the P-element. The digested DNA was size fractionated on a long (15 cm) 0.7%agarose gel and a blot of the size fractionated DNA was probed with labeled plasmid rescue DNA. As is shown in Figure 6, the probe hybridized to larger fragment in the wild type lane than in the mutant lanes. This result indicates that there been a rearrangement between the mutant and wild type in the part of genomic DNA which includes the plasmid rescue sequence.

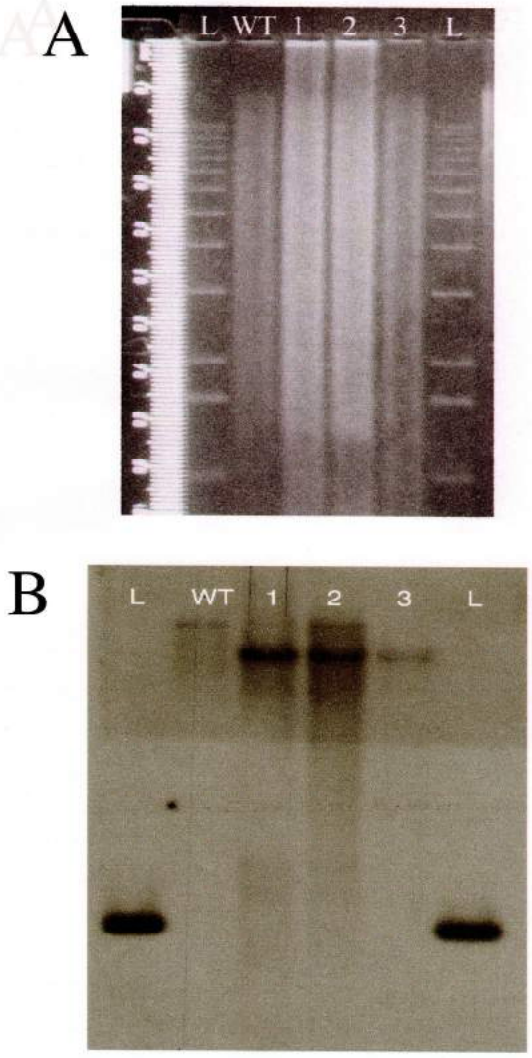

***Figure 6**. Genomic Southern Analysis. A) Agarose gel of SacII digested chromosomal DNA from wildtype (WT) and 10995 (1, 2, 3) flies. B) Autoradiograph of a nylon blot of the gel shown in (A) hybridized with ^{32}P-labeled plasmid rescue DNA from line 10995.*

Northern Analysis

Blots of poly A^+ RNA from wild type and mutant flies were probed with labeled plasmid rescue clone DNA. As shown in Figure 7 the same size signal is detected for both lines. Note that the signal is stronger for the wild type. Since the mutation in the *Mhc* gene is lethal, the flies that are used throughout this work are heterozygous for the mutation. Therefore some normal transcript is made in the mutant flies. Although the class did not perform a loading control test, the importance of loading controls when considering quantitative differences in the amount of DNA or RNA present on a blot or gel was discussed in class.

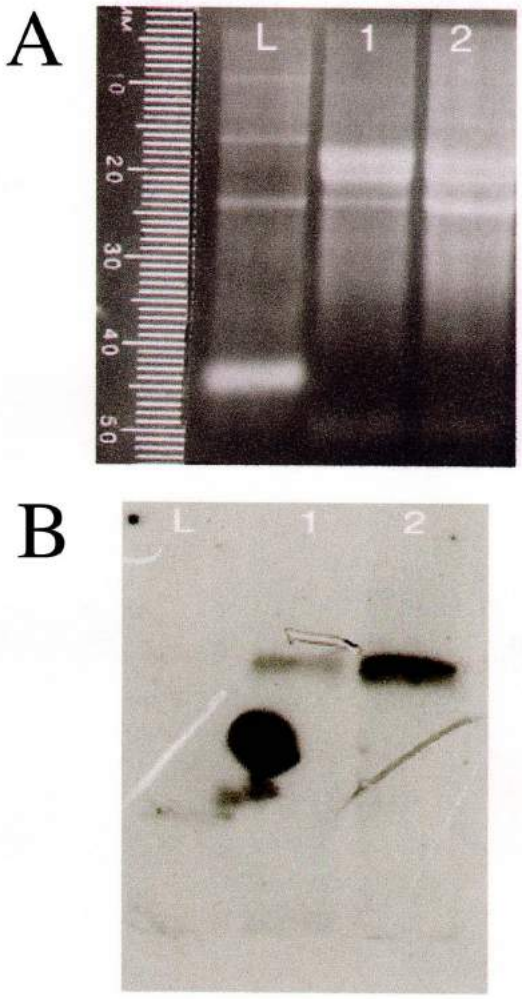

***Figure** 7. Northern Analysis. A) Denaturing formaldehyde agarose gel of Poly A^+ RNA from Line 10995 (lane 1) and wildtype (lane 2) flies. B) Autoradiograph of a nylon blot of the gel shown in (A) hybridized with ^{32}P-labeled plasmid rescue DNA from line 10995.*

cDNA Library Screen and *in vivo* Excision

Because a "spiked" library was used, all of the students recovered the ~6 kb cDNA clone that had previously been isolated from the λ Zap library. An

example of a plaque lift filter hybridized with the biotin-labeled probe can be seen in the Results section of the Course Web Site located at http://www.people.hofstra.edu/beverly_clendening/adv_molecular_bio. The reported sizes of myosin heavy chain transcripts range from 6.2 - 8.2 kb. It is possible, therefore, that the clone the class started with was not full length. The cDNA clone was excised *in vivo* and plasmid DNA was isolated. The plasmid DNA was digested with *EcoRI* to isolate the cDNA insert from the pSK vector. In addition to the *EcoRI* cloning site of the vector, there are also two *EcoRI* sites within the cDNA, one site at each end of the cDNA. A single digest with *EcoRI* produced three fragments, the vector (~ 3 kb), an ~ 1 kb cDNA fragment and an ~ 4.8 kb cDNA fragment. The experiments that the class performed using this cDNA clone were more difficult than the previous experiments since the clone is large and prone to rearrangement. Even when the students are given the previously isolated phage clone, the plasmid they isolated, in some cases, was not full length (~ 6kb). Only full-length clones were used for subcloning into an expression vector.

Subcloning the cDNA into an Expression Vector

The cDNAs of the expected size (~ 6 kb) were subcloned into the *EcoR I* site of the pTrcHis2 expression vector (Invitrogen). Although many colonies were obtained following transformation of One Shot™ cells with the ligated DNA, few contained plasmids of the expected size (~9 kb, ~5 kb insert in the 4 kb vector). The pilot studies for this final experiment were not complete before the first offering of the class, therefore, no subclones were available for distribution. All plasmids obtained by the class that were close in size to the expected plasmid were digested with *XbaI* and *NarI* to determine the orientation of the insert in the vector. There is a recognition sequence for *XbaI* in the multiple cloning site of the expression vector downstream of the *EcoRI* insertion site. The *Mhc* sequence contains no *XbaI* recognition site. There is a recognition sequence for *Nar I* 3.5 kb from the 5' end of the *Mhc* fragment. There are no recognition sequences for *Nar I* in the vector. A diagnostic gel was run to reveal the orientation of each cloned cDNA. If the cDNA was cloned into the expression vector in the correct orientation for expression, the *Xba I/Nar I* digest should yield 1.5 and 7.5 kb (approximate) fragments. If the orientation is reversed 3.5 and 5.5 kb (approximate) fragments will be obtained. Figure 8 shows some of the results of *the Xba I/Nar I* digests of the student's subclones. Lane 6 contains 7.5 kb and 1.6 kb fragments. This approximates the expected size of the fragments for clones oriented in direction needed for expression. Lane 4 has bands at 4.8 and 2.4 kb which does not match the expectation for cloning of the *Mhc* fragment in either direction. Half of the students obtained a similar result. I believe that these aberrant results were caused by a rearrangement within the large subclone.

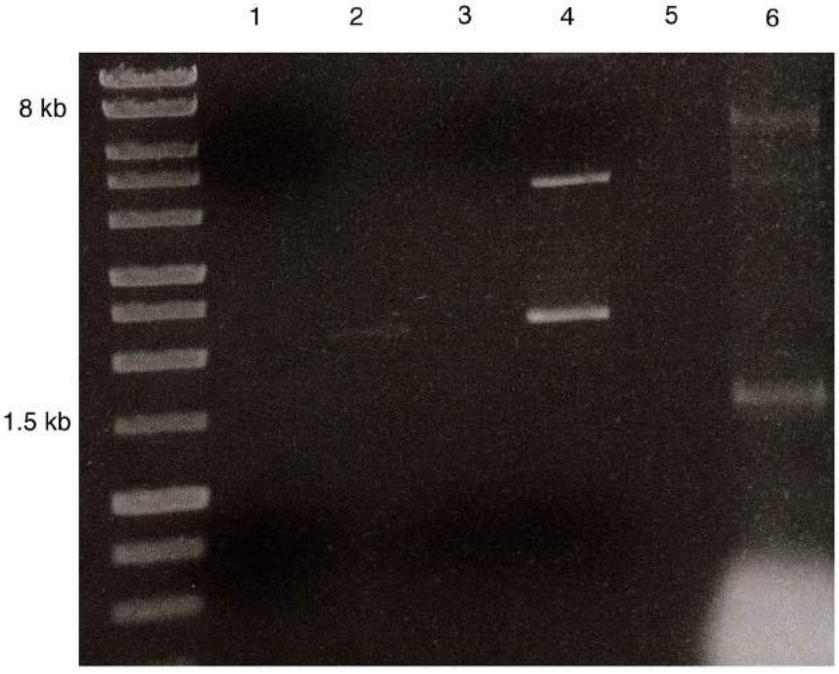

Figure 8. *Agaraose gel of digested subcloned cDNA. Myosin heavy chain cDNA clones that had been subcloned into the pTrcHis expression vector were digested with Xba I and Nar I and fractionated on a 0.8% agarose gel. Lane 4 contains a 2.4 and a 4.8 kb fragment, probably from a truncated cDNA cloned in the opposite orientation from that needed for expression. Lane 6 shows a 1.5 and a 7.5 kb fragment. This subclone is oriented in the vector in the proper direction for expression.*

Expression of the Protein

All groups used the subclone that appeared to of the correct size and in the correct direction for expression (shown in lane 6 of Figure 8). None of the groups obtained a protein product that was similar in size to the 220 kD *Drosophila* myosin heavy chain gene. Most of the students obtained a product that was 31-45 kD (data not shown). It is likely that this is due, at least in part, to incorrect processing of this protein by the bacterial host.

Muscle Proteins. After this simple muscle protein extraction procedure, the major muscle proteins bands can be visualized on an acrylamide gel. Figure 9 shows a gel of the proteins extracted from wild type and line 10995 flies. The amount of myosin heavy chain protein is decreased in line 10995; the amounts of other muscle proteins appear to be unaffected.

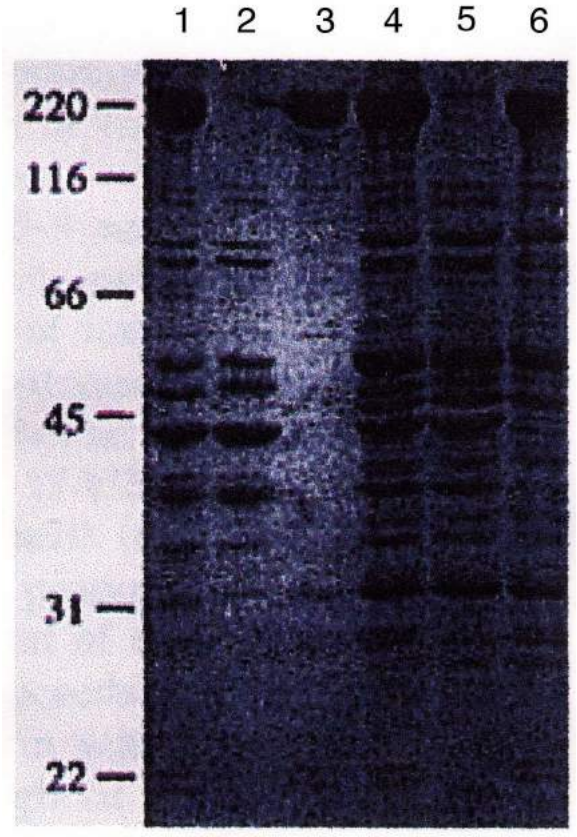

Figure 9. *Polyacrylamide gel of muscle proteins from wild type (lanes 1, 4, and 6) and line 10995 (lanes 2 and 5) flies. The top band in each lane is myosin heavy chain protein; note the decrease in the amount of myosin heavy chain in lanes 2 and 5.*

Alternative Splicing

Drosophila muscle myosin heavy chain protein is encoded by a single copy gene that is alternatively spliced at number of different sites to give a large number of distinct isoforms. One example of this alternative splicing is seen in the exclusion of exon 18 from embryonic and some larval and adult transcript and its inclusion in other larval and adult isoforms message (Rozek and Davidson, 1986). In addition, there are several alternate polyadenylation sites in the *Mhc* gene. We used a simple PCR experiment to demonstrate the alternative splicing of exon 18. cDNA was made from adult and embryonic Poly A^+RNA using oligo dT as a primer. A subsequent PCR reaction using primers spanning exons 17 and 18 produced an 800 bp product from adult samples and no product from embryonic samples (Figure 10). Primers for β actin were used as an internal control for cDNA quality.

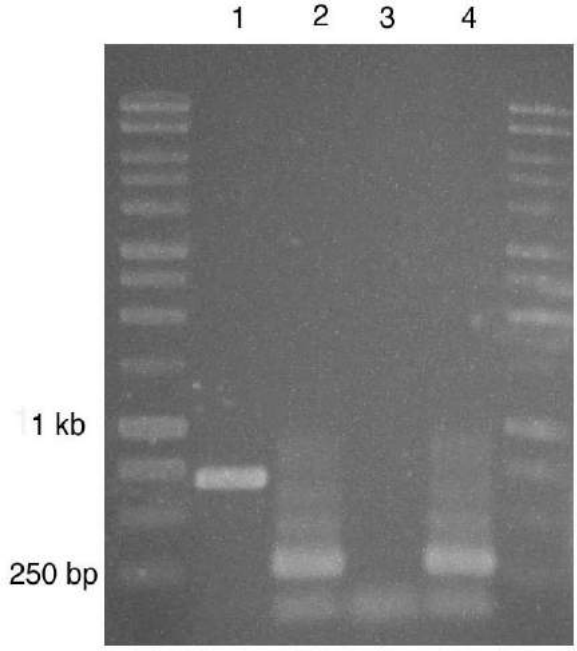

Figure 10. *Agarose gel of RT-PCR products spanning exons 17-18 from adult (lane 1) and embryonic (lanes 3) wildtype flies. Primers for β actin were used as an internal control for cDNA quality (lane 2 adult, lane 4 embryonic).*

Independent Projects

Most groups were able to isolate a plasmid rescue clone from their assigned line of P-insertion flies. These clones were sequenced and the students were able to perform a Blast analysis to locate the P-element insertion within the *Drosophila* genome. Since I do not screen P-element lines for multiple P-element insertion prior to assigning them as projects, it is possible for a group to obtain more than one plasmid rescue clone. I have the students prepare Southern and Northern Blots for their "unknowns" at the same time as they prepare blots for line 10995. Some groups obtain their plasmid rescue clones early enough to prepare probe and carry out the hybridization procedures

DISCUSSION

This is an advanced laboratory course that presumes that the students already have some experience with liquid handling, agarose gels, sterile techniques and other fundamental laboratory skills. It was designed primarily for students who are planning to pursue careers in biology research and biotechnology. The course design is beneficial for these students for a number of reasons. First of all, students who take this laboratory course are exposed to a large battery of molecular biology techniques (Table 1). Secondly, rather than merely exposing students to a set of techniques, the course was designed to mimic the logical progression of a research project where the results of one experiment are the starting material for the next experiment. Moreover, the syllabus mimics research setting in that more than one procedure is carried out in each laboratory session and that students are given more responsibility for preparation and intermediate steps in a procedure than is typical for a standard laboratory course. Finally, the incorporation of an independent research component in the course allows the students to solidify what they have learned and to thoroughly analyze and interpret data that has not been carefully worked out to always give the expected results.

Since this is a laboratory techniques course, assessment of student learning is based primarily on the demonstration of competence in the laboratory. This competence requires not only that the students be able to carry out the laboratory procedures, but also that they understand the concepts discussed in the introductory lectures and the rationale for the laboratory procedures. The students are required to keep a detailed laboratory notebook. All laboratory entries must be dated and identified; all procedures and results must be explained in detail. Where appropriate, results must be tabulated. In addition, a statement of the purpose and rationale for the procedure must accompany all separate procedures within an experiment. The results of all experiments (which may span several laboratory sessions) must be accompanied by a discussion and conclusion entry. I require that students keep their notebooks up-to-date. The students are required to purchase a notebook with removable carbon copies. I collect the notebook carbons after the third class session in order to check entries for accuracy and thoroughness; I do not grade the notebooks at this time. I collect the notebooks for grading at mid- semester. I do not warn the students that I will be collecting notebooks. I also grade the notebooks at the end of the semester. Notebook grades are 40% of the final grade. The students write 3 laboratory reports; one is a report on their individual project. Average grades on the reports constitute 30% of the final grade. Each group of students working together on an "unknown" gives a PowerPoint presentation of their individual project. The grade on this report constitutes 10% of the final grade. Finally, I give an oral final examination to test the students' knowledge of the genetic and biochemical concepts underlying the procedures they have learned. The grade on this exam constitutes 20% of the final grade.

The class as it is presented here was designed for advanced undergraduate and beginning masters' level students and these students have access to the classroom during non-class hours. If the class is offered for lower level students or if the students are not allowed access to the laboratory during non-class times, it would probably be unrealistic to plan to carry out all of the experiments outlined herein.

The class as presented here also requires the availability of a considerable amount of equipment, all of which may not be readily available for use in a student laboratory class at all institutions. The development and equipping of this student laboratory was supported by funds from the National Science Foundation, Department of Undergraduate Education, under the Course, Curriculum and Laboratory Instrumentation Program (NSF-DUE#9980931). Some of the supplies and materials required for this course are quite costly and may prohibit the offering of a similar course at some institutions. Table 2 lists the equipment needed for this course as well as the material and supplies needed that may not be routinely available in undergraduate laboratories. Costs of equipment and supplies are not given as these can vary dramatically over time and are dependent on class size, the vendor and special pricing arrangements.

The difficulties we encountered with the subcloning and expression experiment are likely to be due to the large size of the clone that makes it prone to rearrangement and to the choice of a bacterial expression vector. It is possible that the λ ZAP clone the class started with had a rearrangement. When the clone was first isolated, restriction mapping indicated that it was full length and that no rearrangements were present. Rearrangements may have occurred subsequently. A bacterial expression vector with an epitope tag for easy purification was used. The choice of a bacterial expression system reflects its use in a teaching laboratory. The students are accustomed to working with bacteria; the use of a yeast, insect or mammalian expression system would require that the students learn a new system. In a one-semester course, this is probably not feasible. These more advanced techniques might be performed as a part of a two-semester laboratory or a course designed solely for beginning graduate students. I plan to clone the cDNA into the Invitrogen Echo host-cloning vector so that it can easily be transferred into a bacterial, yeast, insect or mammalian expression vector for this purpose.

Planned Modification

Once the students have obtained the cDNA, it is possible to use the clone to introduce a whole range of more advanced techniques. We attempted to subclone

the cDNA into an expression vector for future expression and purification of the protein. Other possible techniques that could be introduced once the cDNA is obtained include site-directed mutagenesis and *in vitro* transcription for RNase protection studies or *in situ* hybridization. At this time, I do not have plans for incorporating these techniques into my course.

Adaptation of Course for High School Teachers

While this course was originally designed for students who plan to work in a laboratory setting, it is easily modified for other audiences. One of the other audiences this course originally targeted was biology majors who are pre-service secondary school educators. With the prominence of genetic and molecular biology in the news (the human genome project and the use of stem cells serve as examples) and the incorporation of biotechnology into many aspects of medicine, reproductive technology, food production, and the forensic science, there is a push to incorporate an exposure to basic concepts behind genome sequencing, recombinant DNA and other biotechnological advances into the experience of high school students. High school teachers need to understand these concepts thoroughly before they can teach them. Since much of this technology is hard to understand and visualize until it is experienced first-hand, a laboratory course covering recombinant DNA and other molecular biology techniques is becoming more important in the training of high school teachers. The pre-service teachers who are biology majors are encouraged to take this course. In addition, I plan to modify the course so that it can be taught at a less advanced level over a six-week summer session. The target audience for this version of the course will be in-service high school biology teachers. This version of the course will include the introductory computer tutorials, characterization of the mutant, the plasmid rescue and the southern analysis. An independent project will not be included in this course but the class participants would spend time developing lesson plans for incorporating biotechnology into their high school laboratory classes.

Acknowledgement

- Sanford I Bernstein, Ph.D. (San Diego State University) provided a cDNA that was used as a probe to isolate the λ Zap cDNA clone.
- Carol St. Angelo, Ph.D.(Hofstra University) provided valuable assistance in the pilot studies for the course.
- Billy Santoro (Hofstra University undergraduate) converted my ideas for the tutorials into computer programs.
- BIO 139 and 2139 students at Hofstra University helped work out the "bugs" in the course during its first running.
- Stratagene, Inc donated the λZAP cDNA library.
- Development of this course was funded by NSF-DUE CCLI Program, DUE#9980931

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Flies in the Soup and Maggots in the Marmalade: A Laboratory Exercise To Investigate Food Contamination by Insects

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***ABSTRACT*:** We present a classroom exercise that we developed and currently use with non-major biology students. This exercise allows students to investigate insect contamination in common foods and introduces students to food safety guidelines. Each student collects data from three or four different types of food, such as catsup, macaroni and cheese, and paprika by taking samples, isolating suspected contaminants, and examining contaminants using a stereomicroscope. Upon finding suspected contamination, the student makes a slide mount and examines the suspicious material using a compound microscope. This exercise emphasizes investigative and deductive science by pressing students to differentiate between plant cells, animal cells, and inorganic matter. This laboratory exercise is simple, fast, and inexpensive. It also provides a departure point for discussions of food safety, insect pests, pesticide use, sub-sampling, and approximation.

KEYWORDS: insects, food safety, laboratory exercise

Introduction

This article describes a successful laboratory exercise that lets students investigate everyday food products for contamination by insects. We found it successfully conveys the process of investigation, introduces or re-enforces student microscopy skills, and generates rewarding discussions of science, scientific methods, ethics, and consumer protection. In our exercise, students are provided background information from the United States Food and Drug Administration (FDA) concerning allowable levels of food contamination (http://vm.cfsan.fda.gov/~dms/dalbook.html). Students are then provided with samples of everyday food items to investigate for contamination. After the exercise, students calculate their average daily consumption of insect parts.

In the second part of the exercise, students write a short paper discussing the reasons for allowable levels of contamination and trade-offs associated with higher levels of pesticide use. Students weigh the relative costs of potential human illness against the costs of insect management. According to the FDA, anything from a warehouse beetle to *Salmonella* can cause serious illnesses (FDA Consumer, July-August 1991). However, preventing insect contamination can be expensive, both from the direct costs of pesticide application and regulation and from the exposure of humans to potential toxins or carcinogens (http://vm.cfsan.fda.gov/~lrd/fdaact.html).

The comprehensive nature of this exercise allows students to participate in an investigation, practice microscopic techniques, and develop methods for sub-sampling, population estimation, and identification skills. Beyond acquiring these basic laboratory techniques, the exercise can be a departure point for discussions about the abundance and biology of insects and about the tradeoffs between preventing contamination and increasing human exposure to pesticides.

Student Background

Each year, the average American consumer unknowingly eats approximately 2.5 pounds of insect fragments, contaminants found in most fresh and processed foods (Olsen 1991). Because entirely eliminating insect contamination is impossible, the Food and Drug Administration has established guidelines for the number of insect "parts" that are

allowable in retail foods (Wagner 1992). The guidelines are set forth in the "Food Defect Action Level List" in which foods are listed with amounts of contaminants, including insect parts which are allowable because of no known risk to human health (Code of Federal Regulations 1998).

The Food Defect Action Level lists many of our favorite foods. For example, chocolate has up to 60 insect parts allowed per 100 grams, macaroni and cheese boxed dinners have up to 225 parts allowable per 225 grams, and ground cinnamon has up to 400 insect fragments per 50 grams (http://vm.cfsan.fda.gov/~dms/dalbook.html)!

FDA GUIDELINES

The FDA sets the action levels (contamination levels at which food is rejected because contamination levels are unacceptable) because it is economically impractical to grow, harvest, and process raw products that are totally free of insect contamination defects. These "non-hazardous, naturally occurring" defects are often in the form of small pieces of insects that have been incorporated into the food during processing (Code of Federal Regulations 1998).

The FDA guidelines (http://vm.cfsan.fda.gov /~dms/dalbook.html) present the maximum allowable contamination "based on natural or unavoidable defects in foods for human use that present no health hazard". These guidelines are enforced through random inspections of food by FDA laboratories using sub-sampling techniques (Gentry, et al. 1991a, b).

Sub-sampling is used to reduce the time, effort, and expense of examining a food product. Sub-sampling entails an assessment of a random portion of the whole product. It is thoroughly investigated and then the amount of contaminant in that sample is extrapolated to estimate the contaminant in the whole sample. For example, if a jar of grape jelly contains 400 grams and 2 insect parts are found in 1 gram, then the whole jar should contain about 800 insect parts. To ensure accuracy, multiple sub-samples (at least 4) are taken, and the amounts of contamination found in each are multiplied by the total amount of product to obtain an average contamination per amount of product.

Table 1 shows excerpts from the FDA food defect action level report indicating the product, source of insect contamination and significance to the consumer (http://vm.cfsan.fda.gov/~dms/dalbook.html).

In addition to these foods, many other foods contain thresholds for insect contamination. Other products do not have defect levels because of low levels of contamination or because contamination poses no threat. The FDA list provides the following information:

> ***PRODUCTS WITHOUT DEFECT LEVELS***
> "If there is no defect action level for a product, or when findings show levels or types of defects that do not appear to fit the action level criteria, FDA evaluates the samples and decides on a case-by-case basis. In this procedure, FDA's technical and regulatory experts in filth and extraneous materials use a variety of criteria, often in combination, in determining the significance and regulatory impact of the findings.
> The criteria considered are based on the reported findings (e.g., lengths of hairs, sizes of insect fragments, distribution of filth in the sample, and combinations of filth types found). Moreover, FDA interprets the findings considering available scientific information (e.g., ecology of animal species represented) and the knowledge of how a product is grown, harvested, and processed."

Table 1. *Selected food and acceptable contamination levels.*

APPLE BUTTER	Insects	Average of 5 or more whole or equivalent insects (not counting mites, aphids, thrips, or scale insects) per 100 grams of apple butter
	DEFECT SOURCE: *Whole or equivalent insects - preharvest, and/or post harvest and/or processing insect infestation*	
	SIGNIFICANCE: *Aesthetic*	
APRICOTS, CANNED	Insect filth	Average of 2% or more by count has been damaged or infected by insects
	DEFECT SOURCE: *Pre-harvest insect infestation*	
	SIGNIFICANCE: *Aesthetic*	
CHOCOLATE AND CHOCOLATE LIQUOR	Insect filth	Average is 60 or more insect fragments per 100 grams when 6 100-gram subsamples are examined OR Any 1 subsample contains 90 or more insect fragments
	DEFECT SOURCE: *Insect fragments - post harvest and/or processing insect infestation.*	
	SIGNIFICANCE: *Aesthetic*	

MACARONI AND NOODLE PRODUCTS	Insect filth	Average of 225 insect fragments or more per 225 grams in 6 or more subsamples
	DEFECT SOURCE: *Insect fragments - preharvest and/or post harvest and/or processing infestation.*	
	SIGNIFICANCE: *Aesthetic*	
OREGANO, GROUND	Insect filth	Average of 1250 or more insect fragments per 10 grams
	DEFECT SOURCE: *Insect fragments - preharvest and/or post harvest and/or processing insect infestation.*	
	SIGNIFICANCE: *Aesthetic*	
TOMATO PASTE, PIZZA AND OTHER SAUCES	Drosophila fly	Average of 30 or more fly eggs per 100 grams OR 15 or more fly eggs and 1 or more maggots per 100 grams OR 2 or more maggots per 100 grams in a minimum of 12 subsamples
	DEFECT SOURCE: *Pre-harvest and/or post harvest and/or processing insect infestation*	
	SIGNIFICANCE: *Aesthetic*	
WHEAT FLOUR	Insect filth	Average of 75 or more insect fragments per 50 grams
	DEFECT SOURCE: *Insect fragments - pre-harvest and/or post harvest and/or processing insect infestation.*	
	SIGNIFICANCE: *Aesthetic*	

Preventing the defects from insect contamination is not an easy task. In fact pesticides are most often used in a field to either prevent loss of yield (mass per acre) to insect pests or to prevent loss of aesthetic value of a crop. Often the threshold to prevent aesthetic loss is very low because in general consumers avoid agricultural products with any form of damage (holes in the apple, bruised fruit, beans with feeding damage, etc.). During processing, few pesticides can be applied for obvious reasons. The stance of the FDA is evident in the following statement:

> ***USE OF CHEMICAL SUBSTANCES TO ELIMINATE DEFECT LEVELS***
> "It is FDA's position that pesticides are not the alternative to preventing food defects. The use of chemical substances to control insects, rodents and other natural contaminants has little, if any impact on natural and unavoidable defects in foods. The primary use of pesticides in the field is to protect food plants from being ravaged by destructive plant pests (leaf feeders, stem borers, etc.).
> A secondary use of pesticides is for cosmetic purposes--to prevent some food products from becoming so severely damaged by pests that it becomes unfit to eat."

LABORATORY EXERCISE

Materials:

Cheesecloth
10 ml Pipettes
Tweezers, dissecting needle
Funnel
50 ml Graduated Cylinder
Petri Dishes
Electronic Scale (optional)
Stereoscope
Light Microscope
Slides and cover slips
Distilled water
Tablespoon (may be needed to break up jelly)

Suggested food list:

Apple butter
Jelly (any flavor)
Apricot preserves
Paprika
Ketchup
Tomato Sauce

Student Investigation Methods

Jelly and Apricot Preserves*:* Students, who extract contamination from jelly or apricot preserves use cheesecloth, a funnel, a graduated cylinder, tap or distilled water, a pipette, a petri dish, tweezers, a tablespoon, slides and cover slips, stereoscope and microscope. After the materials are collected, the

students set up a simple apparatus to filter the jelly through the cheesecloth. A piece of cheesecloth (approximately 6 inch by 12 inch) is cut and folded over once resulting in a 6 inch by 6 inch piece. This piece of cheesecloth is then placed inside the funnel as a lining. The funnel is placed in a 50 ml graduated cylinder so the amount of filtrate can be measured. After this apparatus is set up, the students measure out about a tablespoon or 3-5 grams of jelly/preserves.

This sample of jelly/preserves can then be placed in the middle of the cheesecloth in the funnel. Water is poured over the jelly while breaking up the jelly using a tablespoon, and the filtrate is collected in the graduated cylinder. This procedure helps to break up the jelly/preserves so the insect fragments can be dislodged from the jelly. After filtering about 40 ml of water the student can discard the jelly/preserves and cheesecloth, saving the water in the graduated cylinder. From the graduated cylinder, the students extract 5 ml samples from the bottom (the exoskeleton of insects sinks) by pipette to a petri dish. The petri dish is then placed under a stereoscope and the student can pick out possible insect fragments. Anything that is extracted from the petri dish should be placed on a slide, covered with a cover slip, and analyzed using a compound microscope.

***Apple Butter**:* Students can use the same technique as with jelly to extract insect fragments from apple butter. Alternatively, students can take a much smaller sample and dilute it with water. To do this procedure, students measure out one gram of apple butter in a Petri dish and add about five milliliters of distilled water. Students stir the sample with a toothpick until water in dispersed throughout. Students can then pick out suspect insect fragments and mount them on slides. These slides are then examined using a light microscope.

***Ketchup/Tomato Sauce**:* Students examining ketchup/tomato sauce will use the alternative technique outlined in the apple butter methods section. Students will measure out about 1 teaspoon or 0.5 grams of ketchup/tomato sauce and place it in a Petri dish. They will then add 5 ml of water so the ketchup/tomato sauce becomes diluted. The students can look through the sample under the stereoscope and place any suspect pieces onto slides to be looked at under the compound microscope.

***Paprika/Spices**:* Paprika and other spices are harder to examine because contamination is difficult to separate from the dry powder. Students should place approximately one gram of the sample in a petri dish and begin by looking at it through a stereoscope. They should use a dissecting needle to sift the sample. Anything that looks suspect should be placed on a slide and looked at under a compound microscope.

***Macaroni and Cheese**:* The macaroni noodles can be placed directly onto a petri dish to be analyzed under a stereoscope. Pasta noodles often have rodent hair that can be seen directly using a stereoscope. The powdered cheese should be investigated for contamination by examining it as with the spices under a dissecting scope.

Results

Students should fill in table 2 with the results of their investigation. They should report how many insect fragments or pieces of contamination they find in each sample. In addition, students can also place their results on the board and analyze the class results.

Table 2. Insect contamination of food examined.

Product	Sample Mass or volume	# Insect contamination	Notes
1.			
2.			
3.			
4.			
5.			
6.			

PART ONE:

The following are thought questions to help students bring closure to the activity.

Answer the Following Questions:

1. Based on you observations of the sub-sample, would any of these products be rejected under the FDA allowable contamination levels?
2. Was there any effect of name brand vs. generic-brand foods? If so, describe the difference.
3. What would you do to improve this investigation of contamination?
4. Are there any products that you will not eat anymore?
5. Why are there allowable insect contamination levels in human foods?

6. What feature allowed you to distinguish between animal and plant contamination of food?
7 Do you think you found all of the insect parts in the food that you sampled?
8. What other methods might you try to better quantify insect contamination?

PART TWO:

The following is an expansion on the exercise to help students hone their analysis and communication skills.

Take-Home Food/ Bug Problem.

So, as new scientists, we were a bit surprised to see you take the 2.5 pounds of insects consumed every year as fact! That seems like a lot of bugs.

First part of the assignment: Calculate how many grams of bug parts you need to eat on average with every meal to eat 2.5 pounds a year. Show all work and all assumptions. So, for example, assume that you eat x pounds of food for breakfast, x for lunch and x for dinner, how many pounds is that for the day? Assume that this is a normal, not leap year. Some useful conversions: 1 kilogram = 2.2046 pounds; 1 ounce = 28.35 grams; 16 ounces = 1 pound.

Second part: So, why do you eat so many bug parts? Please write a 1-2 page essay discussing each of the following questions: Why is food contaminated by bug parts? What are the trade-offs for bug-free food? Why does the Food and Drug Administration allow so many insect parts in the foods you eat? Finally, what is your impression of conducting a lab where you examine food for insect parts? Did it change the way you looked at last night's meal?

INSTRUCTOR NOTES

Foods chosen:

Based on the textures and types of food (e.g. powdered cheese vs sticky jelly) chosen or assigned, students will use different techniques to investigate food for insect contamination. Insect contamination is the most difficult to discover in finely ground food products like flour and cocoa and in solid foods like candy bars. Other products such as frozen broccoli and soups are also difficult for students to investigate because of the large size of the sample (broccoli spears) or the dark color and thick nature of the broth (most soups).

In this article we have presented the foods that we use in our laboratory exercises and in which students have been very successful at discovering contamination. Name brand and generic brands of food were included, which allowed our students to create hypotheses and conduct investigations to determine if there was any difference in contamination with name brand and generic products. We developed two fairly simple qualitative methods for separating insect contamination from food products. For upper division students, quantitative methods can be easily adapted by precisely measuring sample volume or mass, conducting repeated measures, and calculating the total contamination based on the results of the sub-sampling.

Typical Results:

Figure 1 shows a typical outcome of this activity in a class of 24 students. Every group found at least some contamination. The range varied from one part per sample to nine parts per sample. These results were consistent in that products like cheese and apricot preserves are easier to sort for contamination than the paprika and jelly. In addition to finding contamination, students had to distinguish between insect contamination and plant parts. Rarely are large parts or whole insects found in a sample because consumers react poorly to finding a recognizable insect part in their food. Nevertheless, whole small insects are occasionally found. Figure 2 shows a mite that was discovered in grape jelly. Students learn to distinguish plant cells from insect parts by detecting cell walls. Figure 3 shows the contrast between an insect part and a plant cell wall from samples obtained in ketchup.

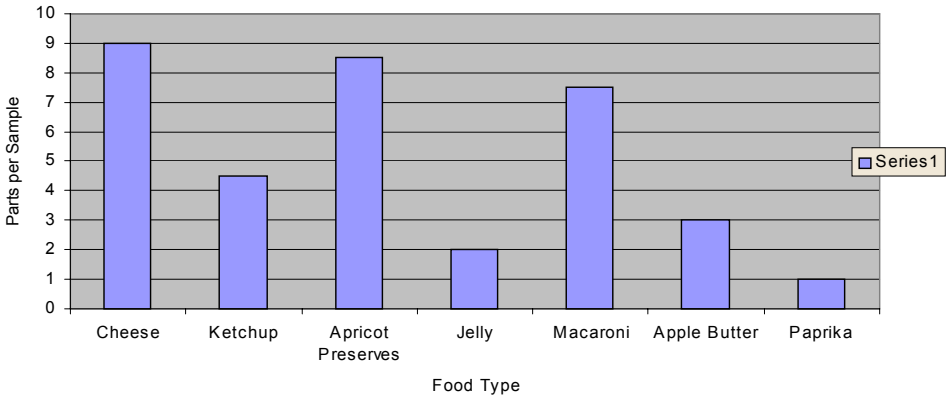

Figure 1. *Average number of insect contamination per sample found in seven different food types. Each sample was less than one gram but not measured to an exact weight. The powdered cheese had the most contamination per sample for this class.*

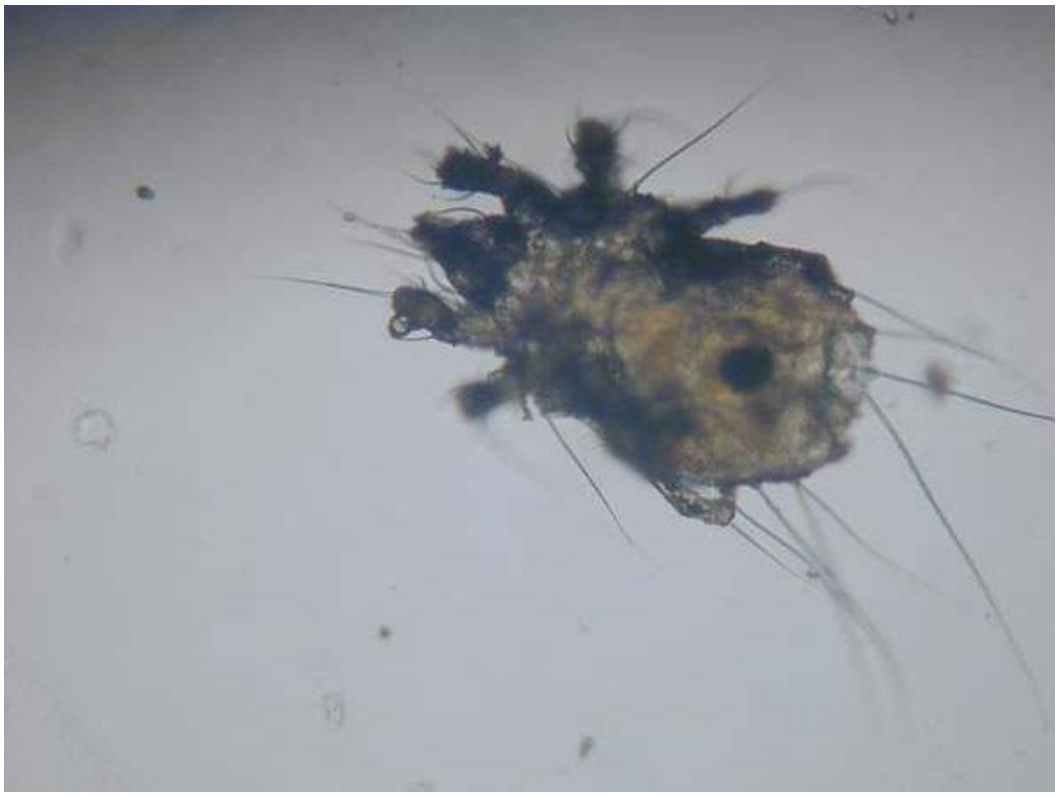

Figure 2. *A mite, found in grape jelly.*

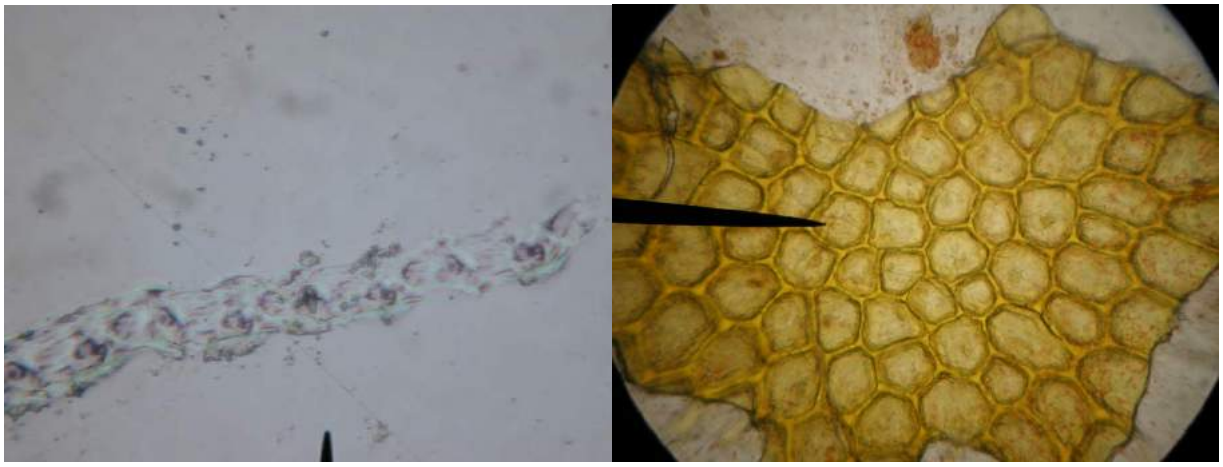

Figure 3. *Insect contamination (left) and plant part (right). Students can distinguish plant from animal cells based on the presence of cell walls.*

Student Results for the Take-home Portion.

The calculation of insect parts consumed at each meal relates the activity to student's lives and allows them to become an active participant in the evaluation. It also re-enforces the principals of sub-sampling and the role of approximation in calculating an average insect consumption based on human consumption of products. For example, what estimate did students use for the amount of food consumed per meal? Students could even extend the exercise by bringing in a meal or two and weighing it. For simplicity, our students usually estimate that each meal averages 1 pound.

After doing the exercise, students often re-examine their beliefs about eating insect contamination. Potential health risks associated with human pesticide exposure are frequently in the news, and many students are aware of some of these debates. In addition, large amounts of information are available on the World Wide Web, and there is lots of controversy. For example, in the late 1980s chlordane contamination (a pesticide used to control termites) was estimated to affect inhabitants of up to 75% of U.S. homes. In 1999 Lindane was banned on apples because it was potentially carcinogenic. Most recently (2000), the chemical chlorpyrifos, an organophosphate used to control mosquitoes, cockroaches, fruit and vegetable crop pests, and lawn pests, was banned because of potential neurological damage to children. The ban has been highly controversial with several scientists arguing that exposure to insects capable of transmitting pathogens is more dangerous than exposure to the pesticide. For more information on chlorpyrifos, consult http://www.soton.ac.uk/~jrc3/chudler/pest.html.

As a continuation of this exercise and if time allows, students can be assigned the paper by Peterson and Shurdut (1999). This presents a relatively non-technical (suitable for non-majors) paper on health risks associated with cockroaches versus those associated with pesticide. Discussion can then focus on such questions as the type of insect contamination and its consequences (i.e. eating a flour beetle is a lot less risky than eating a cockroach) and the risk from pesticide exposure (a relatively large contingent of organic farmers present information on the World Wide Web).

CONCLUSIONS

This laboratory exercise is an important part of and Introductory Biology course. We have found students to be very engaged in this activity because they are excited to explore the food they are eating and are interested in learning about why insect parts are allowable contamination. This activity is open-ended and students enjoy being in charge of their own investigation. In fact, we have found that students seldom want to stop at three different food types and that they are excited to share their findings with fellow classmates (a reaction different from when they see their cheek cells for the first time using a microscope).

We believe that this activity can be successfully used for almost any non-majors or in the basic biology class and can be adapted further to meet advanced level class requirements. Although the main goal in developing this exercise was to implement and practice scientific investigation, learn about sub-sampling, and become familiar with the use of a microscope, we have found that the student interest and excitement carries beyond these goals. The outcome of this laboratory has led to animated discussions of food safety, insect biology, and risk assessment and regardless of their final opinion, we find that students never think of the food they eat in the same way again.

Acknowledgements

This project was developed as part of the undergraduate independent research requirement in the Department of Biology. We thank the department for its support and especially Julie Shaffer, Charlie Bicak, and Leon Higley for useful comments on earlier versions of this manuscript.

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ACUBE
Web Site
http://acube.org

The Association of College and University Biology Educators (ACUBE), placed the organization's rich archive of materials online for the benefit of the members and interested biology educators. Nearly 45 years of the society's publications and resources are currently accessible.

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Biocomplexity in Undergraduate Education: From Hard Data to Hard Decisions

A BioQUEST Curriculum Consortium Workshop

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Explore new ideas.
Share teaching strategies.
Discuss new classroom approaches with professional peers.
Create biocomplexity problem solving experiences for students, and investigate BioQUEST software.

When: June 15-23, 2002
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Cost: The workshop, funded by the **Howard Hughes Medical Institute**, provides workshop development, private campus rooms, and all meals.

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ACUBE 2002
Columbia College Chicago, Illinois
September 12-14, 2002

Columbia College Chicago is located in downtown Chicago, in the south Loop area, at the hub of America's heartland, and easily accessible by air, rail and road. Columbia is within walking distance of the lakefront, and three world-class museums; the Field Museum of Natural History, the John G. Shedd Aquarium, and the Art Institute of Chicago. Columbia College Chicago is itself the home of the Museum of Contemporary Photography, and numerous other museums, cultural and shopping venues are readily accessible via public transit or automobile. Because of our downtown location, Columbia College Chicago can be conveniently reached using private vehicles, or public transportation from anywhere in the metropolitan area, in particular from O'Hare International Airport and Midway Airport.

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The primary objectives of the department are to provide students with a comprehensive scientific and mathematical background, the adaptability and flexibility they will need in order to evolve with continuing changes in the world, and the ability to deal effectively with specific changes in their professional disciplines. Thus, critical thinking and problem solving are major objectives of the various departmental programs.

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ACUBE 46TH Annual Meeting

September 12-14, 2002

Columbia College
Chicago, IL

Visualizing and Communicating Environmental Issues

Preliminary Program

Thursday, September 12th

6:00 - 8:00 PM	**Registration and Reception**
8:00 - 9:00 PM	**Opening Session**

Welcome to ACUBE:
ACUBE President: Malcolm Levin, *University of Illinois – Springfield*

Welcome to Columbia College:

Program Chair: Bob Wallace, *Ripon College*
Local Arrangements Chair: Abour Cherif, *Columbia College*

OPENING ADDRESS (Public Welcome to Attend)

9:15 - 10:15 PM	**Executive Committee Meeting**

Friday, September 13th

7:00 AM - 5:00 PM	**Registration table**
7:00 - 8:00 AM	**Buffet Breakfast** (by Interest Group)
9:00 AM - Noon	**SUSTAINING MEMBER EXHIBITS** (refreshments provided)
8:15-9:45 AM	**CONCURRENT WORKSHOP SESSIONS I**

9:50-10:20 AM **POSTER SESSION I**

10:30 AM - noon **CONCURRENT WORKSHOP SESSIONS II**

10:30 - 11:15 AM **CONCURRENT PAPER SESSIONS I**

11:20 - 12:05 AM **CONCURRENT PAPER SESSIONS II**

12:15 - 1:00 PM **Luncheon and First Business Meeting**
First and Final Call for Nominations!!

1:00 - 1:45 PM **Luncheon Program**

2:00 - 5:00 PM **SUSTAINING MEMBER EXHIBITS**
(refreshments provided)

2:50 - 3:20 PM **POSTER SESSION II**

3:30 - 5:00 PM **CONCURRENT WORKSHOP SESSIONS III**

5:05 - 5:45 PM **Web Committee Meeting**

6:00 - 7:00 PM **Social**
(resumes of candidates available for review)

7:00 - 9:00 PM **BANQUET and Second Business Meeting**
(two-minute speeches prior to banquet; balloting after dinner presentation)

Dinner Presentation

Saturday, September 14th

7:30 - 8:45 AM **Buffet Breakfast** (by Interest Group)

7:45 - 8:45 AM **Bioscene Editorial Board**
,

9:00 - 9:45 AM **CONCURRENT PAPER SESSION IV**

10:00 - 10:45 AM **CONCURRENT PAPER SESSIONS V**

11:00 AM - 12:15 PM **Luncheon and Third Business Meeting**
BUSINESS MEETING
Election Results:
Lynn Gilley, Elmira College
Resolutions:
Dick Wilson, Rockhurst University
Executive Secretary Report:
Pres Martin, Hamline University
Bioscene:
Ethel Stanley, Beloit College & .Tim Mulkey, Indiana State University
Presidential Address:
Malcolm Levin, SIU-Springfield
2003 Meeting:

12:30 - 1:15 PM **Steering Committee Meeting**
Includes newly elected Steering Committee members!

Call for Abstracts

Association of College and University Biology Educators (ACUBE)
46th Annual Meeting
Columbia College – Chicago, Illinois
Thursday, September 12 – Saturday, September 14, 2002

Visualizing and Communicating Environmental Issues

Paul Ehrlich and John Holdren proposed that a powerful tool for understanding environmental issues is through a very simple equation,

Human Impact = Population x Affluence x Technology (I = P x A x T)

How can the perspectives of the arts, communications and media, the social sciences and others regarding these factors inform biology and biologists in identifying problems and reaching solutions?

Presentations and workshops addressing other topics are welcome. Below are some examples of potential presentations:

- Curricula – Environmental Studies/Environmental Sciences – content/method/delivery
- Sampling and Reconstructing Environments: What the past and the present tell us
- Imaging/Digital Video/GIS as Forms of Communication
- Environmental Ethics
- Teaching Identification/Taxonomy: why/how
- Interdisciplinary Problem Solving
- Global Change/Global Change Research: State of the World, 2002

Many of you have addressed these issues in creative ways. Please consider sharing your ideas and techniques at the ACUBE 46th Annual Meeting at Columbia College in Chicago in 2002.

Please email your abstract AND mail a hard copy of the abstract with the completed form BEFORE June 1, 2002 to:

Robert Wallace, Department of Biology, 300 Seward St., Ripon College, Ripon, WI 54971
Ph: 920-748-8760 Fax: 920-748-7243 email: WallaceR@Ripon.edu

Proposed Title:_____

Presentation type _____ 90 minute workshop _____ 45 minute paper _____ Poster

Equipment/facility needs:
_____35 mm slide projector _____Overhead projector
_____Macintosh projection system _____Macintosh computer lab
_____PC projection system _____PC computer lab
_____Other: (explain)

Name of presenter:_____

Work address of presenter: _____

_____

Phone No. presenter:_____ email_____

Please include names and contact information for additional presenters on back.

Women In Life Science Disciplines

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ABSTRACT: The breakdown of the life science disciplines within the College of Life Sciences and Agriculture at the University of New Hampshire allowed us to establish the graduation patterns of men and women within the respective disciplines. From 1994 - 1999, women earned more bachelor degrees than men in five of the eight life science disciplines and constituted 68% to 88% of the graduates in Animal Science, Nutritional Science, and Zoology. Such findings are only possible because the "biological/agricultural," "biosciences," "natural sciences," or "life sciences" are broken down into individual components and hence provide a more nearly accurate representation of gender patterns in these disciplines.

KEY WORDS: LifeSciences/Women, Natural Sciences, Biosciences, Baccalaureate degrees in Life Sciences/Women.

INTRODUCTION

The number of women receiving baccalaureate degrees has been on the rise for three decades (National Science Board (NSB), 2000; U.S. Department of Education, 1999, 2000; National Science Foundation, 1997). Although data indicate that women account for 53.56% of all of the four-year degrees granted in the United States from 1986-1996, women earned only 44.34% of the bachelor degrees in the natural sciences during that same time period (NSB 2000). However, natural sciences, as defined by the National Science Board, includes all physical, earth, atmospheric, oceanographic, biological and agricultural sciences. Since there is considerable variation among scientific disciplines, how the sciences are clustered in a study may impact the outcome when considering the persistence and success rates of women. A more definitive description of the natural sciences, by individual disciplines, would provide more information about the enrollment patterns and percentages of women in a specific natural science discipline.

To date, most studies have combined the science disciplines together when reporting the success and retention rates of women. Several publications have cited barriers, under-representation, low persistence rates, lack of role models, lack of recognition, and stereotyping of women in Sciences, Mathematics, and Engineering (Dujari, 2000; Morrell and Andrews 1998; Rayman and Brett, 1995; Seymour, 1992 a, b; 1995 a, b; Ware, Steckler and Leserman, 1985).

Rosser (2000) sought to narrow the scientific field when she specified that women now receive 50% of the undergraduate degrees in the biological/agricultural sciences (p. 11) and 52.6% in biological sciences (p. 17). Rosser stated that "large numbers of women are attracted to the various life science disciplines because these disciplines help fulfill their desire to help people" (Rosser p. 3).

It is difficult to clearly delineate the status of women in a particular science because the terms "natural sciences," "life sciences," and "biosciences" are broad and contain a considerable variation of science disciplines. A breakdown by discipline would provide a more accurate representation of women in a particular scientific area. This is demonstrated in the NSB 2000 findings which list the biological/agricultural sciences (BAS) as a separate discipline of the natural sciences. Total head counts indicate that women slightly outnumbered men in BAS in 1996 (NSB 2000) (Table 1).

Table 1. *Earned Baccalaureate Degrees in the Natural Sciences in the U.S. by Gender. Source: National Science Board. Science and Engineering Indicators: 2000*

Years	Majors	Total (%)	Men (%)	Women (%)
1993	Natural Sciences	77,312 (100.00)	42,316 (54.73)	34,996 (45.27)
	Biological/Agricultural	59,621 (100.00)	30,439 (51.05)	29,182 (48.95)
	Physical Science	14,188 (100.00)	9,424 (66.42)	4,764 (33.58)
	Earth/Atmospheric/ Oceanographic Sciences	3,503 (100.00)	2,453 (70.03)	1,050 (29.97)
1994	Natural Sciences	83,791 (100.00)	45,600 (54.42)	38,191 (45.58)
	Biological/Agricultural	65,268 (100.00)	33,347 (51.09)	31,921 (48.91)
	Physical Science	14,655 (100.00)	9,588 (65.42)	5,067 (34.58)
	Earth/Atmospheric/ Oceanographic Sciences	3,868 (100.00)	2,665 (68.90)	1,203 (31.10)
1995	Natural Sciences	90,845 (100.00)	48,474 (53.36)	42,371 (46.64)
	Biological/Agricultural	71,470 (100.00)	35,915 (50.25)	35,555 (49.75)
	Physical Science	14,897 (100.00)	9,605 (64.48)	5,292 (35.52)
	Earth/Atmospheric/ Oceanographic Sciences	4,478 (100.00)	2,954 (65.97)	1,524 (34.03)
1996	Natural Sciences	98,322 (100.00)	51,766 (52.65)	46,556 (47.35)
	Biological/Agricultural	78,469 (100.00)	39,100 (49.83)	39,369 (50.17)
	Physical Science	15,396 (100.00)	9,694 (62.96)	5,702 (37.04)
	Earth/Atmospheric/ Oceanographic Sciences	4,457 (100.00)	2,972 (66.68)	1,485 (33.32)
Total	Natural Sciences	350,270 (100.00)	188,156 (53.72)	162,144 (46.28)
	Biological/Agricultural	274,828 (78.46)	138,801 (50.50)	136,027 (49.50)
	Physical Science	59,136 (16.88)	38,311 (64.78)	20,825 (35.22)
	Earth/Atmospheric/ Oceanographic Sciences	16,306 (4.66)	11,044 (67.73)	5,262 (32.27)

The present study examines the enrollment trend of men and women in eight of the disciplines of the College of Life Sciences and Agriculture (COLSA) at the University of New Hampshire. The University of New Hampshire is a public land, - sea - , and space-grant University serving an undergraduate population of about 11,000 students (59% women, 41% men).

COLSA, one of seven colleges within the University, provides a blend of the basic and applied aspects of the life sciences and agriculture. It consists of eight life science disciplines which include Animal Science, Biochemistry, Biology, Microbiology, Natural Resources, Nutritional Science, Plant Biology, and Zoology and three social science disciplines consisting of Adult and Occupational Education, Environmental Resource Economics, and Community Development. Only data from the eight life science disciplines are included in this study.

OBJECTIVES

The objectives of this study are:

1. to establish the discipline(s) of the life sciences most or least selected by women and men at the University of New Hampshire,
2. to compare the percentage of earned baccalaureate degrees in each of the life science concentrations by gender, and
3. to compare University life science data with national statistics.

METHOD

All University data in this study were obtained from the Office of Institutional Research, University of New Hampshire. Absolute headcounts of males and females were used since this is the manner in which national data are compiled. The percentages were rounded out to two decimal places and, due to rounding error, may not sum exactly to one hundred percent.

DATA and DISCUSSION

As noted previously, nationally, more women than men earn baccalaureate degrees (NSB 2000). Nevertheless, this is not the case when examining the natural sciences collectively. Data in Table 1 demonstrate that from 1993 to 1996, approximately 7.44% more men than women overall were awarded baccalaureate degrees in the natural sciences (NSB 2000).

However, the gender disparity is markedly reduced when the biological/agricultural sciences

(BAS) are differentiated from the natural sciences. Overall, men earned 50.5% and women 49.5% of the baccalaureate degrees granted in BAS disciplines from 1993-1996 (NSB 2000) (Table 1). Furthermore, women (39,369) slightly outnumbered men (39,100) in BAS in 1996 (NSB 2000) (Table 1).

The fact that women attained parity with men in BAS (1996) suggests that women may indeed outnumber men in certain disciplines of the biological/agricultural sciences. Examination of the earned bachelor degree pattern within COLSA, by gender, could determine those life science disciplines enrolled in by men and women collectively and separately.

Life Science Discipline Selection by both Genders from 1994-1996

Of the 2,017 men and women COLSA graduates from 1994-1999, 484 (24%) matriculated in Biology, 468 (23.20%) in Natural Resources, 360 (17.85%) in Animal Science, 193 (9.57%) in Nutritional Science, 161 (7.98%) in Microbiology, 158 (7.83%) in Zoology, 127 (6.3%) in Biochemistry, and 66 (3.27%) in Plant Biology (Table 2).

Biology, Natural Resources, and Animal Science were the disciplines most selected by students. Together, these three disciplines accounted for 1,312 (65.05%) of the 2,017 bachelor degrees awarded in the life sciences during the six year period. Collectively, the other five disciplines, Nutritional Science, Microbiology, Zoology, Biochemistry, and Plant Biology constituted 34.95% of the degrees earned by both genders during the same time period.

Although these data provide insights as to the enrollment trends of both genders collectively within the life sciences, a breakdown by gender is essential to establish those disciplines most selected by men or women.

Life Science Discipline Selection by Women

Of the 1,187 women graduates from 1994-1999, in the eight life science disciplines of COLSA, 271 (22.83%) received baccalaureate degrees in Biology, 248 (20.89%) in Animal Science, 204 (17.19%) in Natural Resources, 170 (14.32%) in Nutritional Science, 108 (9.10%) in Zoology, 90 (7.58%) in Microbiology, 64 (5.39%) in Biochemistry and 32 (2.70%) in Plant Biology (Table 2).

Biology, Animal Science, and Natural Resources were the three disciplines selected by 60.91% of the women followed by Nutritional Science and Zoology which, together, enrolled 23.42% of the women. Microbiology, Biochemistry, and Plant Biology combined were selected by 15.67% of the female graduates (Table 2).

Life Science Discipline Selection by Men

Of the 830 men granted baccalaureate degrees in the life sciences during the same time period, 264 (31.81%) were in Natural Resources, 213 (25.66%) in Biology, 112 (13.49%) in Animal Science, 71 (8.55%) in Microbiology, 63 (7.59%) in Biochemistry, 50 (6.02%) in Zoology, 34 (4.10%) in Plant Biology and 23 (2.77%) in Nutritional Science (Table 2).

Natural Resources, Biology, and Animal Science comprised 70.96% of the baccalaureate degrees granted to men followed by Microbiology, Biochemistry, Zoology, Plant Biology and Nutritional Science which, together, comprised 29.03% of the male degree recipients (Table 2).

Male and Female Distribution by Life Science Discipline

Total head count comparisons of both genders together, within individual disciplines, at the University of New Hampshire, indicate that women outnumber men in
Animal Science, Biochemistry, Biology, Microbiology, Nutritional Science, and Zoology. Men outnumber women in Natural Resources and Plant Biology (Table 2).

Since there are more women (1,187) than men (830) in COLSA, it is not surprising that, numerically, women outnumber men in six of the eight life science disciplines. One might argue that these results are biased in favor of women given that women outnumber men in these disciplines. However, if one compares the percentage of men within each science discipline, with respect to total number of men enrolled in COLSA, one might note that the gender enrollment trend reverses in three of the eight disciplines (Biochemistry: males 7.59%, females 5.39%; Biology: males 25.66%, females 22.83%; Microbiology: males 8.55%, females 7.58%). Although these gender enrollment reversals are evident, the percentage differences are negligible. Consequently, to be consistent with national studies, we report our findings using total head counts.

Gender Representation by Discipline

Of the 193 graduates in Nutritional Science, 170 (88.08%) were women and 23 (11.92%) were men, constituting a 7:1 female to male ratio in this discipline. Likewise, of the 360 earned degrees in Animal Science, 248 (68.89%) were conferred to women and 112 (31.11%) to men, constituting more than a 2:1 woman to man ratio in this discipline. Additionally, of the 158 degrees granted in Zoology, 108 (68.35%) were granted to women while 50 (31.65%) were to men, again constituting more than a 2:1 female to male graduate ratio. (Table 2).

Since women also slightly outnumbered men in Biology, Biochemistry, and Microbiology, no gender predominance is discernable in these disciplines. (Table 2) This trend is also apparent if one considers the mean number of degrees earned during this same time period (Fig. 1).

TABLE 2. EARNED BACCALAUREATE DEGREES IN THE COLLEGE OF LIFE SCIENCES AND AGRICULTURE (COLSA) BY MAJOR AND GENDER

	LIFE SCIENCE DISCIPLINES							
	Animal Sciences # (%)	Biochemistry # (%)	Biology # (%)	Natural Resources # (%)	Microbiology # (%)	Nutritional Sciences # (%)	Plant Biology # (%)	Zoology # (%)
1994								
Total	58 (100.00)	20 (100.00)	43 (100.00)	66 (100.00)	15 (100.00)	22 (100.00)	6 (100.00)	34 (100.00)
Males	14 (24.14)	9 (45.00)	22 (51.16)	33 (50.00)	6 (40.00)	3 (13.64)	4 (66.67)	11 (32.35)
Females	44 (75.86)	11 (55.00)	21 (48.84)	33 (50.00)	9 (60.00)	19 (86.36)	2 (33.33)	23 (67.65)
1995								
Total	61 (100.00)	25 (100.00)	101 (100.00)	67 (100.00)	19 (100.00)	36 (100.00)	10 (100.00)	21 (100.00)
Males	23 (37.70)	13 (52.00)	46 (45.54)	36 (53.73)	7 (36.84)	5 (13.89)	4 (40.00)	5 (23.81)
Females	38 (62.30)	12 (48.00)	55 (54.46)	31 (46.27)	12 (63.16)	31 (86.11)	6 (60.00)	16 (76.19)
1996								
Total	52 (100.00)	28 (100.00)	91 (100.00)	84 (100.00)	33 (100.00)	25 (100.00)	12 (100.00)	21 (100.00)
Males	16 (30.77)	14 (50.00)	41 (45.05)	42 (50.00)	19 (57.58)	2 (8.00)	8 (66.67)	6 (28.57)
Females	36 (69.23)	14 (50.00)	50 (54.95)	42 (50.00)	14 (42.42)	23 (92.00)	4 (33.33)	15 (76.19)
1997								
Total	74 (100.00)	13 (100.00)	76 (100.00)	74 (100.00)	37 (100.00)	32 (100.00)	16 (100.00)	22 (100.00)
Males	23 (31.08)	4 (30.77)	33 (43.42)	44 (59.46)	18 (48.65)	4 (12.50)	8 (50.00)	5 (22.73)
Females	51 (68.92)	9 (69.23)	43 (56.58)	30 (40.54)	19 (51.35)	28 (87.50)	8 (50.00)	17 (77.27)
1998								
Total	56 (100.00)	23 (100.00)	94 (100.00)	113 (100.00)	31 (100.00)	37 (100.00)	11 (100.00)	24 (100.00)
Males	19 (33.39)	12 (52.17)	34 (36.17)	73 (64.60)	12 (38.71)	6 (16.22)	4 (36.36)	8 (33.33)
Females	37 (66.07)	11 (47.83)	60 (63.83)	40 (35.40)	19 (61.29)	31 (83.78)	7 (63.64)	16 (66.67)
1999								
Total	59 (100.00)	18 (100.00)	79 (100.00)	64 (100.00)	26 (100.00)	41 (100.00)	11 (100.00)	36 (100.00)
Males	17 (28.81)	11 (61.11)	37 (46.84)	36 (56.25)	9 (34.62)	3 (7.32)	6 (54.55)	15 (41.67)
Females	42 (71.19)	7 (38.89)	42 (53.16)	28 (43.75)	17 (65.38)	38 (92.68)	5 (45.45)	21 (58.33)
Total	**360 (100.00)**	**127 (100.00)**	**484 (100.00)**	**468 (100.00)**	**161 (100.00)**	**193 (100.00)**	**66 (100.00)**	**158 (100.00)**
Males	**112 (31.11)**	**63 (49.61)**	**213 (44.01)**	**264 (56.41)**	**71 (44.10)**	**23 (11.92)**	**34 (51.52)**	**50 (31.65)**
Females	**248 (68.89)**	**64 (50.39)**	**271 (55.99)**	**204 (43.59)**	**90 (55.91)**	**170 (88.08)**	**32 (48.48)**	**108 (68.35)**
Total Life Sciences	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)	2,017 (100.00)
Total Disciplines	360 (17.85)	127 (6.30)	484 (24.00)	468 (23.20)	161 (7.98)	193 (9.57)	66 (3.27)	158 (7.83)

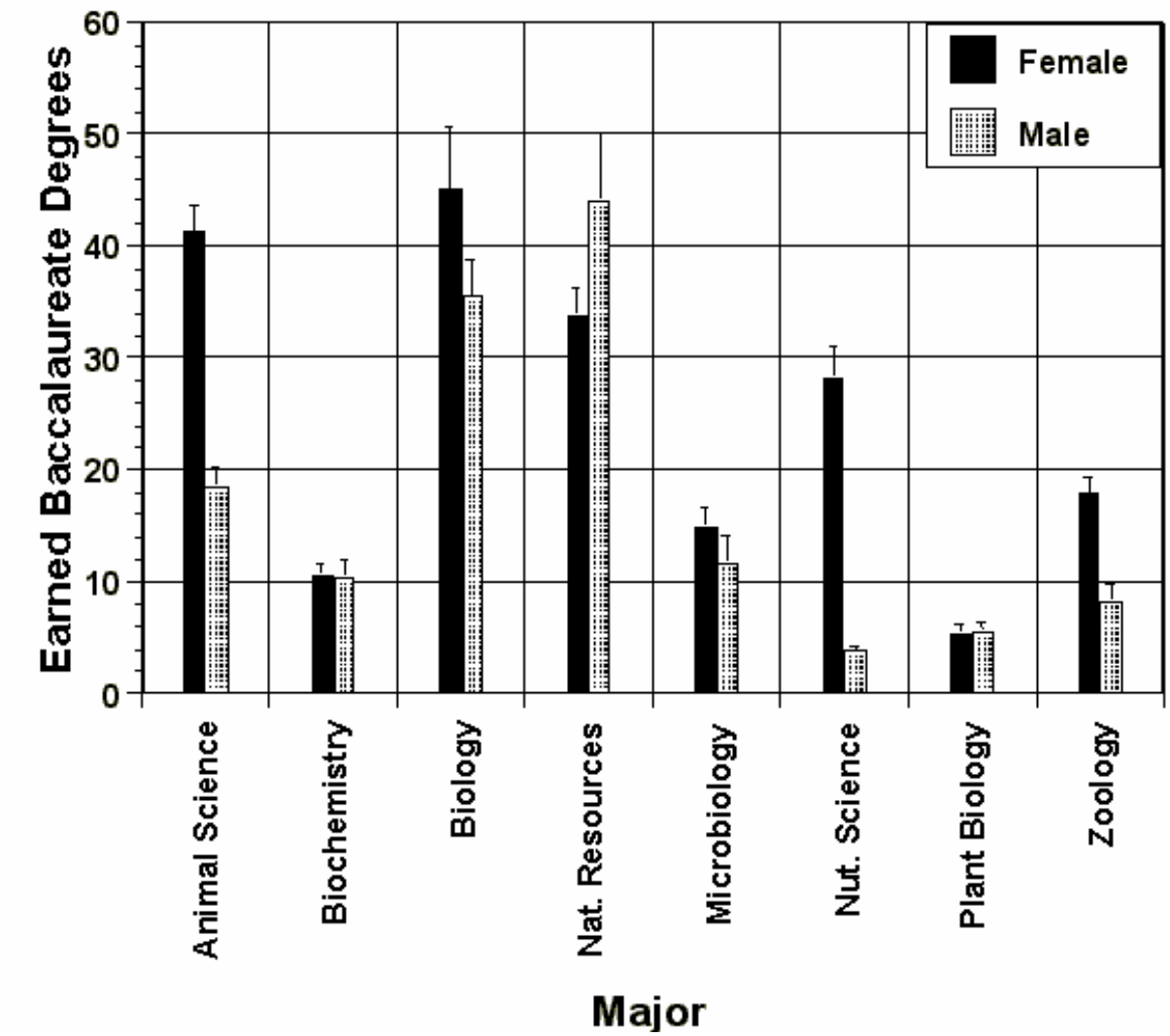

Figure 1. *Average Number of Earned Baccalaureate Degrees in Life Science Disciplines (COLSA) (1994-1999). Error bars represent +1 standard error of the mean.*

As presently indicated, men outnumbered women in Natural Resources (264 men/204 women). Both genders were nearly equally represented in Plant Biology (34 men/32 women) (Table 2). No obvious gender predominance is detected in these fields of study.

Earned Life Science Degrees in the United States

The U.S. Department of Education reports (1999, 2000) break down the various sciences into specific disciplines (Table 3). Data from these reports indicate that, nationally, in 1996-1997 and 1997-1998, women earned more baccalaureate degrees than men in Animal Science, Biology, Microbiology, Nutritional Science, and Zoology (Table 3). In contrast, these same reports show that men attained more bachelor degrees than women in Biochemistry/Biophysics, Natural Resources, and Plant Science (Table 3).

Although, nationally, women were more highly represented in the same five life science disciplines as in COLSA, differences do exist when considering the percentage of women in each of these life science disciplines (Table 4). Furthermore, the breakdown of the life sciences into specific disciplines, both on the national level and at the University, will establish whether our findings conform with similar national data and permit us to compare our findings with national data that cluster all the life sciences together under the category of biological/agricultural sciences.

Table 3. *Earned Life Science Degrees in the United States (1997-1998) Sources: U.S. Dept. of Education, Digest of Education Statistics. 1999, 2000.*

Years	Science Area	Total (%)	Males (%)	Females (%)
1996-1997	Animal Science (General)	3,183 (100)	1,449 (45.52)	1,734 (54.48)
1997-1998		3,394 (100)	1,480 (43.61)	1,914 (56.39)
1996-1997	Biochemistry/Biophysics	3,708 (100)	1,997 (53.86)	1,711 (46.14)
1997-1998		3,838 (100)	2,014 (52.48)	1,824 (47.52)
1996-1997	Biology (General)	46,632 (100)	20,924 (44.87)	25,708 (55.13)
1997-1998		47,054 (100)	20,536 (43.64)	26,518 (56.36)
1996-1997	Microbiology/Bacteriology	2,417 (100)	1,169 (48.37)	1,248 (51.63)
1997-1998		2,781 (100)	1,345 (48.36)	1,436 (51.64)
1996-1997	Natural Resources (General)	5,511 (100)	3,006 (54.55)	2,505 (45.45)
1997-1998		5,687 (100)	3,039 (53.44)	2,648 (46.56)
1996-1997	Nutritional Sciences	456 (100)	89 (19.52)	367 (80.48)
1997-1998		463 (100)	98 (21.17)	365 (78.83)
1996-1997	Plant Science (General)	396 (100)	263 (64.41)	133 (33.59)
1997-1998		387 (100)	253 (65.37)	134 (34.63)
1996-1997	Zoology (General)	2,800 (100)	1,368 (48.86)	1,432 (51.14)
1997-1998		2,866 (100)	1,312 (45.78)	1,554 (54.22)
	All Fields	166,261 (100)	81,900 (49.26)	84,361 (50.74)

Table 4. *Comparison of COLSA Life Science Discipline Degrees with National Data*

Life Science Disciplines	National Degrees Women (%)	COLSA Degrees Women (%)	Differences (%)
Animal Science	55.44	68.89	13.45
Biochemistry (Biophysics)	46.83	50.39	3.56
Biology	55.75	55.99	0.24
Microbiology (Bacteriology)	51.64	55.91	4.27
Natural Resources	46.01	43.59	-2.42
Nutritional Sciences	79.66	88.08	8.42
Plant Science (Biology)	34.11	48.48	14.37
Zoology	52.68	68.35	15.67

*Percentages are based upon earned degrees over a two year period nationally and a six year period at the University (Tables 2-3).

COLSA Graduation Trends and National Trends

When considering the life sciences collectively, women, overall, earned 50.74% of the life science baccalaureate degrees awarded in the United States in 1997-1998 (Table 3). A similar "clustering" of the biological/agricultural sciences (BAS) indicate that women received 50.17% of the BAS undergraduate degrees in the nation in 1996 (Table 1). These findings suggest that women slightly outnumber men in the life sciences and have attained parity with men in BAS. However, analysis of the degree trends by gender within the individual life science disciplines (which undoubtedly contain many BAS disciplines) indicate that women, on the national level and at the University, outnumber men in Animal Science, Biology, Microbiology, Nutritional Sciences, and Zoology (Table 4). Furthermore, although women were more highly represented than men in these five disciplines,

the predominance of women in the Nutritional Sciences is strongly evident both nationwide and at the University (Table 4).

On a percentage basis, COLSA graduates a considerably higher number of women in Animal Science, Nutritional Science, and Zoology than reported nationally (Table 4). As previously stated, women at the University predominantly enroll these life science disciplines. No explanation is available as to why this is the case.

Nationally, fewer women (46.83%) than men receive undergraduate degrees in Biochemistry/ Biophysics (Table 3) whereas at the University women earn 50.39% of the degrees in Biochemistry (Table 4). While this may not represent a significant difference, this disparity might be explained by the fact that nationally Biophysics is included with Biochemistry while this is not the case at the University. Biophysics is not offered at the University.

Natural Resources and Plant Science (Biology) are the two life science disciplines which enroll more men than women both at the national and University levels (Table 4). No major difference is observed between national and COLSA enrollment trends in Natural Resources (Table 4).

Although women also earned fewer baccalaureate degrees than men in Plant Biology, at the University, on a percentage basis, the number of women in this field was noticeably higher than national statistics (Table 4). This could be due to the low and near equal enrollment of both men and women in Plant Biology in COLSA.

CONCLUSION

This study demonstrates that, nationally and at the University of New Hampshire, women are not only highly represented in the life sciences, but also demonstrate impressive persistence and success rates. Such findings are not discernible if earned baccalaureate data are aggregated into the "biological and agricultural sciences," "biosciences," "life sciences" or "natural sciences" collectively.

While the success of women in specific life science disciplines is noteworthy, it is also important that these women succeed at the next career levels so as to be positioned to serve as role models for other women.

Further studies are needed to determine the representation of women at other colleges and universities throughout the nation. Once this is determined, women can become aware of their accomplishments in the life sciences which may encourage them to venture more confidently into other fields of science where women are still underrepresented.

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Call for Nominations

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ACUBE members are requested to nominate individuals for the office of President-Elect and two at large positions on the ACUBE Steering Committee. Self-nominations are welcome.

If you wish to nominate a member of ACUBE for a position, send a Letter of Nomination to the chair of the Nominations Committee:

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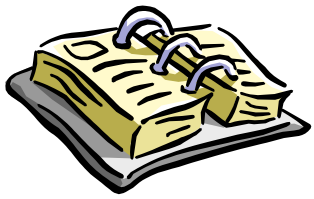

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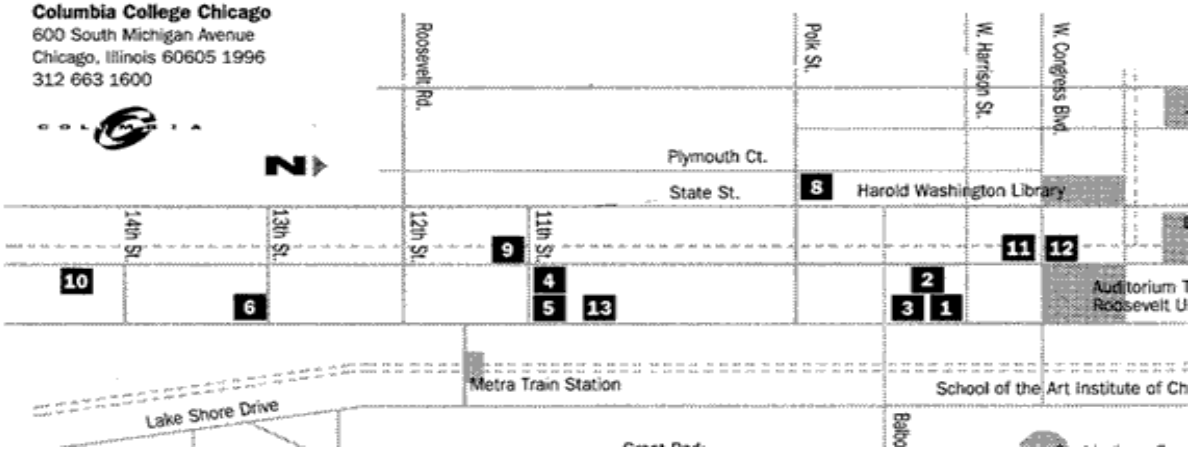

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Please submit all manuscripts to editor(s):

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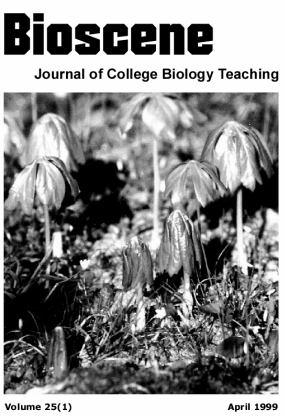

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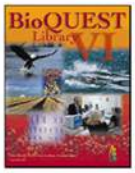

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