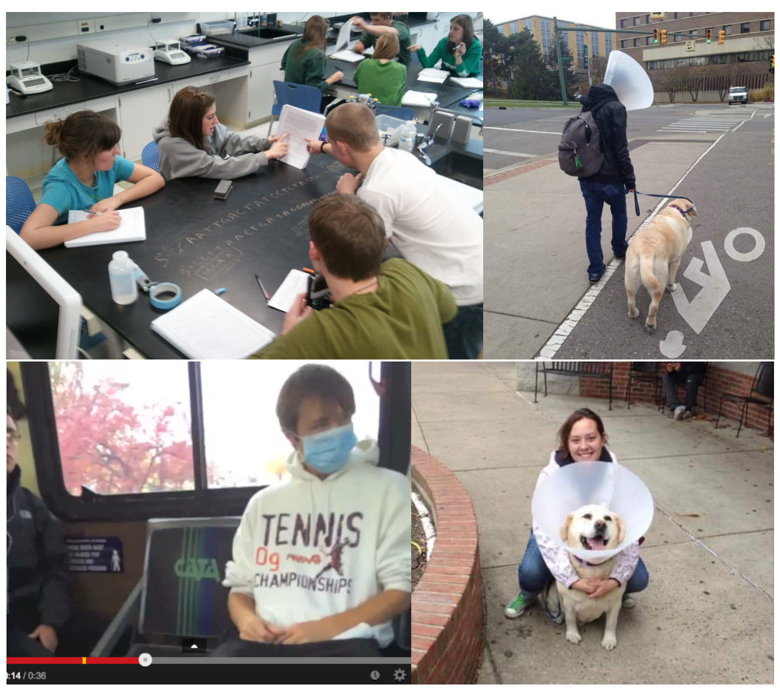
Biology I
LB-144
Cell and Organismal Biology



Lecture & Lab
Fall 2022
(for student in Douglas Luckie's sections)

This publication is a compendium of work authored by:
Candace Igert, Angela Wright, Cori Fata-Hartley, Michael Haenisch, Susannah Cooper, Marjia Krha, Joseph Maleszewski, John Wilterding, Mimi Sayed, and Douglas Luckie

Contents

WELCOME TO LB-144 **(fill in page numbers for yourself)**

- CONTRACT ___
- LECTURE COURSE SYLLABUS ___
- LAB COURSE SYLLABUS ___ & ___
- CHAPTER CHECKLISTS! ___

LABORATORY GUIDE

- GROUP ROLES ___
- WORKING EFFECTIVELY IN SMALL GROUPS ___

RESEARCH STUDIES

- PROJECT: GETTING STARTED ___
- GENERAL PRIMERS ___
- HYPOTHESIS SCORE CARD ___ & ___

WRITING INFORMATION

- INSTRUCTIONS TO AUTHORS ___
- SAMPLES: STUDENT PAPERS ___
- GRADING RUBRICS ___

APPENDIX

- HANDOUTS ___
- PROF OR LA INTERVIEWS ___
- DRACONIAN CONTRACT ___

STUDENT LABORATORY NOTEBOOK

Welcome to LB-144

Welcome to LB 144! The experience you are about to have in the cell & organismal biology course may turn out to be unlike most other lectures/labs that you will have while as an undergraduate. There will be a major emphasis on team effort in this class and your ability to work in a team is crucial to your success in this course and beyond. As a team, you and your partners, will work together to solve biological problems in lecture, discuss scientific ideas in recitation, and pursue research projects within the lab to find answers to the unique questions your group will have posed.

Molecular/Cell/Organismal biology is quickly becoming an integral part of science and society. With new discoveries and ongoing discussions of topics such as: cloning, genetically modified foods/organisms, DNA fingerprinting, genomics, bioinformatics, gene patenting (the list goes on) it becomes crucial to connect it all to the cellular and organismal levels.

Cell & Organismal biology is not an easy subject to master. There are complex concepts as well as a great deal of factual information. Nevertheless, the difficulty of this subject adds to its appeal. The staff of LB 144 will work hard to help you glean the information necessary to achieve in this course; however, your hard work is the most essential element to success.

This experience will without a doubt be exciting, frustrating, and almost overwhelming at times – but it is all with purpose. We hope that you will come out of this course with not only a better understanding of molecular biology, but also a better understanding of the scientific method itself.

We look very forward to working with you throughout the semester and if you have questions please do not hesitate to contact any one of the TAs, GAs, or the Professor. Good luck and enjoy the semester!

- The 144 Staff

144 Contract (sign & return)

A syllabus is a form of contract between the instructor and the students. If you, the student, complete tasks with a specific score a predefined grade is awarded. Read the announcements below and the syllabus *in full* before signing and submitting this page.

1. WORKLOAD *As Undersigned student, I am aware the lecture & lab course is worth 4 credits and will require me to work outside of class 8-12 hours each week. Some weeks will require less, and some weeks will require more effort. If I prepare poorly for class, learning will take longer.*

2. TOURISM *I am aware that I will work with a group of students that sit together in lecture, work together as a research team in lab, meet and study together outside of class at night and on weekends. This course is designed for full-time LBC students and if I need to travel off-campus or off-grid frequently, my group members may become very unhappy, and I should discuss this.*

3. MANY READINGS *As the Undersigned student, I am aware that I will have many required reading assignments each week, and unless I read the assigned pages, answer questions, take notes and study them prior to class, it's likely I will become lost in lecture.*

4. QUIZZES *As the Undersigned student, I am aware that I will have quizzes or graded exercises each week, and unless I read the assigned pages in the reading, take notes and study them prior to the quiz, it's likely I will get a low score on said quiz or exercise.*

5. EXAMS *As the Undersigned student, I am aware midterm exams may be purely essay style and provided in advance, and in this case I should work with my group studying the questions and developing excellent answers in the time prior to the test. If I just "cram" my studies and work into 48 hours prior to the exam, it's likely I will get a low score on said midterm.*

6. UNIVERSITY GRADING SCALE *I am aware this course uses a university scale with higher expectations than high school, excellent work is a 3.0, much more is necessary for a 4.0.*

7. GROUP GRADES *I am aware that I, with the help of other students in my research group, will be authoring one research paper (with a number of drafts) and my grade may include both the score of my sections as well as the score for the work as a whole. I realize I will be expected to review the entire project before submission. If this doesn't work well for me, I should discuss it with my group or the prof immediately.*

8. HONOR CODE *In the authoring of assignments, I accept that any piece of work may be submitted to http://turnitin.com for screening. I am aware that if the work authored by me is found to be plagiarized, I will be given a zero for the assignment & perhaps for LB144 course grade.*

I have read the above announcements and syllabus. I understand the expectations are high but I'm up to the challenge. I agree to the tenets of this contract.

____

Printed Name *Signature* *Date*

144 Contract (keep)

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____

Printed Name *Signature* *Date*

HYBRID EDITION

LB-144: CELL & ORGANISMAL BIOLOGY

TOP HAT *course: app.tophat.com/e/104419*, ZOOM *classroom is: 934-1774-2369*

If you strive to think and communicate like a scientist in your work throughout the semester, in the end, you will be a scientist and really know biology (Think like a serious scientist, not like a pretend one).

LECTURER

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LEARNING ASSISTANTS

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COURSE WEBSITE **http://ctools.msu.edu/144** (mirror => cf.psl.msu.edu/144)

TEXTBOOK (online-only, for Luckie's LB144 custom textbook, http://store.trunity.com/)
"Integrating Concepts in Biology" by Campbell, Heyer & Paradise, 2020 Edition, Trunity Holdings Inc

COURSE PACK "LB-144 Course Pack," (Luckie) from MSU Library Services via local bookstore

INTERNET 20+Mbps (download): 10+Mbps (upload) best. Use D2L, Zoom, TopHat, CATME, Turnitin.

LB-144 = LB-144 (LECTURE) & LB-144L (LAB) OVERVIEW OF CLASSES

There are two overreaching goals in these two connected classes.

1.) To gain a fundamental comprehension of the cellular & organismal processes of life and an appreciation why it is important to understand these processes.
2.) To learn how to think like a scientist and be able to adaptively negotiate a question or problem.

The cell & organismal biology course is an exploration of life at all levels. It examines the interplay of genes, cells, and chemistry allowing organisms to live, survive, and interact with each other and the environment. Specifically, we will study genes (molecular biology), living cells inside organisms (cell biology), organisms and their environments (ecology), genetic variation and inheritance (genetics), and the interactions of the environment and genetics over time (evolution) that led to the diversity of life observed on the planet today.

Our lecture will meet twice a week as two 80-minute class meetings. Once a week in-person (live, synchronous) and once on TopHat (recorded, asynchronous). In this class, you will have daily homework and in lecture we will review and discuss, in depth, parts of the readings. We will examine how scientists perform their work to help you master the ability to ***Think like a serious scientist, not like a pretend one***.

WORKLOAD

The first semester of Biology is a 4-credit course (LB144) that consists of two connected classes (lecture 3 credits, laboratory 1 credit) and because it is two classes it requires twice as many hours of work as one class. For any university-level course, for each credit, you are expected to spend 2-3 hours/week outside of class studying and working on homework assignments. There will be a certain amount of preparation that you will need to do before each lab and readings that you will need to complete (with notes taken) before each lecture. Come to lecture and lab well-prepared or mastering the new material may take much longer than necessary.

SCHEDULE
Both the lecture schedule and the lab schedule are found in the syllabi pages. We reserve the right to modify the schedule if necessary. You will be given advance warning if the schedule needs changed.

OFFICE HOURS & JCLUB
Held each week on Mondays 12:40-2:00pm in-person in our classroom, also you can make appointments.

ACADEMIC HONESTY
Turnitin.com will allow you to review writing assignments prior to submission. If you are caught cheating, you will be assigned a “0” for the assignment or the entire course. The policy for academic honesty at LBC is available online -> https://lbc.msu.edu/advising/academic-policies.html

GRADING
Your grade in this course (LB144) is based on the total percentage earned in the both the lecture portion and the laboratory portion of the course, each worth half. The course will be graded on a flat scale.

4.0= 90-100%   3.5= 85-89.9%   3.0= 80-84.9%   2.5= 75-79.9%   2.0= 70-74.9%   1.5= 65-69.9%   1.0= 60-64.9%   0.0= <60

A “3.0” score is considered Excellent. It is impressive work, top of the class, and the work was done extremely well but nothing beyond what was expected.
A “3.5” is Most Excellent. Every detail of the work was done extremely well, and they found additional papers and evidence beyond what they were told.
A “4.0” is Outstanding. It has the 3.0, 3.5-level elements + student impresses instructor with how much/well they did the work. They taught Prof something.

Late Policy: Assignments are due in lab/lecture at the beginning of the session indicated (at time of entering room) unless otherwise specified. If an assignment is 1 day late, 1 point will be deducted from the final score. After this 24 hour grace period, the penalty becomes more severe: 20% off for two days late, 30% off for three days and so on. After 5 days, you will receive a “0” for the assignment.

Rejected Manuscripts/Reports: Each time a paper is “rejected”, because it did not follow the *Instructions to Authors*, 1 point is deducted. This is independent of the Late Policy, both can occur.

Blind grading: Whenever possible we will score assignments "blind" and thus ask you to not list your name but your "B-PID" (found on D2L). This helps eliminate bias and make grading more fair.

*__Formal written grade appeal process__: If you feel that your assignment was not graded properly, you must submit your concern via appeal in writing (on paper, not via email). You must concisely explain why you object to the assigned grade and what elements of your work in fact demonstrate you mastered the material. Please be advised that if you submit a formal grade appeal about one element of an assignment, we always re-grade your entire exam, paper or quiz and the score may increase, decrease or stay the same. For group assignments, all authors must sign the written request since re-grading may impact all. How well you provide your claim, evidence and reasoning will be assessed, and students who provide good logical arguments supported well by solid relevant evidence will earn approval (you may cite pages of textbooks, or even better, published research papers). Avoid emotional arguments that blame others or arguments based on hearsay, e.g. “I heard from a student” “A TA told me this was correct.” If you do not make logical arguments or provide thoughtful evidence to support them, your appeal will not likely gain traction to be approved. All discussion concerning score changes must be completed within 7 days from the date the grade was officially posted (on the returned assignment or online). No grade changes will be considered after this time. If illness or other emergency prevents you from completing assignments on time, you must make arrangements with your instructor before the due date (example appeal provided in course pack).

LB144 Biology Learning Goals

1. Practice at doing and communicating science. Speak your thoughts smartly.

a) Communicate Scientific Thoughts: Manifest your smart thinking in the best words possible.
   1. Speaking: a high priority of this course is for you to practice public speaking & listening.
   2. Reading: practice careful reading of papers, identification of points, interpretation of figures.
   3. Writing: practice composition of text, writing manuscripts, building data figures and graphs.

b) Design and Analyze Experiments: Make a hypothesis, design experiments, make predictions. Interpret data collected, look for patterns, ways to best share and represent findings.

2. Study the Biology Idea of "Information". Learn examples and mechanisms to store/transmit information at molecular, cell, organismal, population levels.

These **"content"** goals are for you to understand, describe, and give examples of how:

a) Heritable information (like DNA/genes) provides for continuity of life and non-heritable information (like talking) is also transmitted within and between biological systems.

b) Imperfect information transfer, like during reproduction of cells, chromosomes, and genes, leads to *variation* of traits among individuals. (e.g., some beach mice have light colored fur because a mutation in a gene makes it difficult for their hair cells to make dark hair pigment)

c) Interactions among organisms and the environment determine *individual* survival and reproduction. (e.g., animals who are in cooperative groups and communicate live longer)

d) Selection (and other mechanisms) acts on individuals and leads to the evolution of *populations*. (e.g., beach mice with fur that matches the color of sand live longer than others because?)

e) Information in DNA => becomes (transcribed) information as RNA => becomes (translated) information as proteins (e.g. How viruses enter our cells, take control, & make viral proteins)

f) Small simple chemicals can associate to form nucleotides, amino acids, lipids, carbohydrates; which can polymerize, form structures and functions we define as "alive" (life on earth).

3. Practice Transfer of Learning: Work with your group to intentionally transfer knowledge learned in one context (e.g. squirrels) to another new context (e.g. humans).

a) Reflect: Develop personal learning goals and regularly reflect on your progress during the semester. (e.g. regularly consider "What I am supposed to be learning here? Have I mastered that? What about transfer? Can I link this to life on Mars, or humans, or something different?")

b) Collaborate: Confidently cooperate in teamwork, and practice team building, communication and leadership. (e.g. "that's a good idea, should we also test if it works in another animal?" "Jon, you haven't spoken much, what do you think?")

HYBRID EDITION

LB-144: CELL & ORGANISMAL BIOLOGY (LECTURE)

SCHEDULE: Each week= TOP HAT online video lecture & *LIVE in-person lecture*

	Date	*Scale/Level*	*Readings (emphasis)*	*Instructors LIVE or TopHat*
W1	W, 31 **Aug.**	Ecological	Lect.1, Ch18 (18.1 crickets call)	*LIVE in-person (Luckie & LAs)*
W2	online	Ecological	Lect. 2, Ch18 (18.1 frogs sing)	Online videos (Luckie & LAs)
	W, 7 **Sep.**	Ecological	Lect. 3, Ch18 (18.3 corals settle)	*LIVE in-person (Luckie & LAs)*
W3	online	Population	Lect. 4, Ch17 (17.1&.2 fireflies)	Online videos (Chris Paradise)
	W, 14	Population	Lect. 5, Ch17 (17.2 storm petrel)	*LIVE in-person (Luckie & LAs)*
W4	online	Population	Lect. 6, Ch17 (17.3 meerkats)	Online videos (Chris Paradise)
	W, 21	Population	Lect. 7, Ch17 (17.3 continued)	*LIVE in-person (Luckie & LAs)*
W5	M, 26	***EXAM I***	*LIVE in-person (in classroom)*	
	W, 28	Organismal	Lect. 8, Ch16 (16.1 blood BP)	*LIVE in-person (Luckie & LAs)*
W6	online	Organismal	Lect. 9, Ch16 (16.1 sandworts)	Online videos (Chris Paradise)
	W, 5 **Oct**.	Organismal	Lect. 10, Ch16 (16.3 vaccines)	*LIVE in-person (Luckie & LAs))*
W7	online	Organismal	Lect. 11, Ch3 (3.1 Mendel)	Online videos (Luckie & LAs)
	W, 12	Organismal	Lect. 12, Ch3 (3.1 Mendel/SBE)	*LIVE in-person (Luckie & LAs)*
W8	online	Organismal	Lect. 13, Ch3 (3.3 division)	Online videos (Malcolm Campbell)
	W, 19	Organismal	Lect. 14, Ch3 (3.4 mitosis)	*LIVE in-person (Luckie & LAs)*
W9	online	Organismal	Lect. 15, Ch3 (3.5 mitosis)	Online videos (Malcolm Campbell)
	W, 26	Organismal	Lect. 16, Ch3 (3.5 meiosis)	*LIVE in-person (Luckie & LAs)*
W10	M, 31	***EXAM II***	*LIVE in-person (in classroom)*	
	W, 2 **Nov**.	Cellular	Lect. 17, Ch2 (2.1 RNA types)	*LIVE in-person (Luckie & LAs)*
W11	online	Cellular	Lect. 18, Ch2 (2.3 Translation)	Online videos (Malcolm Campbell)
	W, 9	Cellular	Lect. 19, Ch2 (2.4 NCBI)	*LIVE in-person (Luckie & LAs)*
W12	online	Molecular	Lect. 20, Ch1 (1.1, 1.2 Griffith)	Online videos (Luckie & LAs)
	W, 16	Molecular	Lect. 21, Ch1 (1.4 DNA struct)	*LIVE in-person (Luckie & LAs)*
W13	online	Molecular	Lect. 22, Ch1 (1.5 epigenetics)	Online videos (Malcolm Campbell)
	W, 23	Molecular	Journal Club	*LIVE in-person (Luckie & LAs)*
Thanksgiving Break, Nov. 24-27				
W14	online	Molecular	Lect. 23, Ch4 (4.1 Evolution)	Online videos (Malcolm Campbell)
	W, 30	Molecular	Lect. 24, Ch4 (4.2 Miller)	*LIVE in-person (Luckie & LAs)*
W15	online	Molecular	Lect. 25, Ch4 (4.3 competition)	Online videos (Malcolm Campbell)
	W, 7 **Dec**.	Molecular	Lect. 26, Ch4 (4.4 store energy)	*LIVE in-person (Luckie & LAs)*

TBA see official schedule (tentative time/date) **FINAL EXAM** finals week 2022

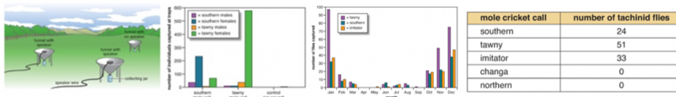
Readings

Ecological System—Information & Environment: Communication within a species, between species, and to exploit other species.

Have organisms evolved to exploit communication between individuals of other species? (Prey Detection)

- (18.1 crickets call)

Cricket songs are exploited by natural enemies

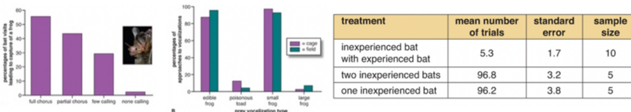


mole cricket call	number of tachinid flies
southern	24
tawny	51
imitator	33
changa	0
northern	0

Raj Ulagaraj, T. Walker: Phonotaxis of crickets in flight: attraction of male and female crickets to male songs, *Science* 182(4118):1278, 1973.

- (18.1 frogs sing)

Frog choruses attract some predators



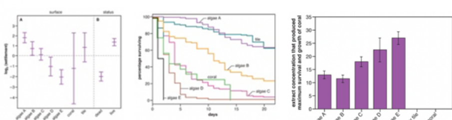
treatment	mean number of trials	standard error	sample size
inexperienced bat with experienced bat	5.3	1.7	10
two inexperienced bats	96.8	3.2	5
one inexperienced bat	96.2	3.8	5

Rachel Page, M. Ryan: Social transmission of novel foraging behavior in bats: frog calls and their referents, *Curr Biol* 16(12):1201-1205, 2006.

Is chemical communication used to block competition or defend self? (Competition)

- (18.3 corals settle)

Information is used by corals during settlement



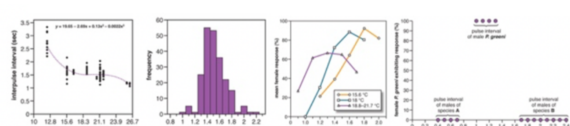
Lindsay Harrington, Katharina Fabricius, et al: Recognition and selection of settlement substrata in corals, *Ecology* 85(12):3428-3437, 2004.

Populations—Behavior & Exchange: Non-heritable information transfer in individuals, imperfect transfer produces variation.

How is information transmitted between members of animal species? (Populations, Communication, Animal Behavior)

- (17.1-2 fireflies blink)

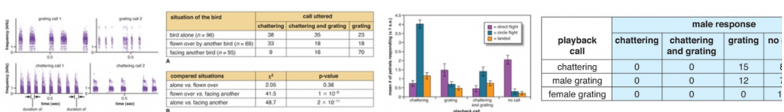
Simple communication in a firefly



Sara Lewis, Michaelidis C, Demary K: Male courtship signals & female signal assessment in fireflies, *Behavioural Ecology* 17:329-35, 2006.

- (17.2 storm petrels call)

More complex communication in a bird



situation of the bird	call uttered		
	chattering	chattering and grating	grating
bird alone ($n = 96$)	38	35	23
flown over by another bird ($n = 69$)	33	18	18
facing another bird ($n = 95$)	9	16	70

A

compared situations	χ^2	p-value
alone vs. flown over	2.05	0.36
flown over vs. facing another	41.5	1×10^{-9}
alone vs. facing another	48.7	2×10^{-11}

B

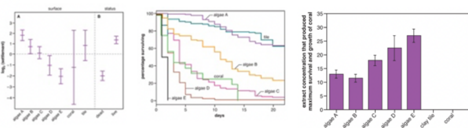
playback call	male response			
	chattering	chattering and grating	grating	no
chattering	0	0	15	8
male grating	0	0	12	7
female grating	0	0	0	1

Vincent Bretagnolle: Calls of Wilson's storm petrel: functions, sexual recognitions and geographic variation, *Behaviour* 111:98-112, 1989

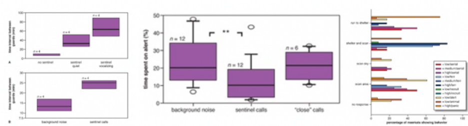
Does group living require more derived mechanisms of information transfer? (Social behavior, Eusociality)

- (17.3 meerkats bark)

Signals and information transfer are used in social animals



Marta Manser: Response of foraging group members to sentinel calls in suricates, *Suricata suricatta*, *Proc Biol Sci* 266(1423):1013-1019, 1999.



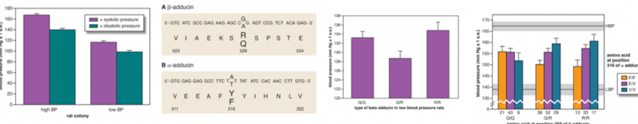
Manser M, Bell M, Fletcher L: The information that receivers extract from alarm calls in suricates, *Proc Biol Sci* :268:2485, 2001.

Organisms—Individual Variation to Group Evolution: Life evolves in altered environment, variation by several mechanisms.

What causes individual variation? (Variation and Population Genetics)

- (16.1 rat blood pressure)

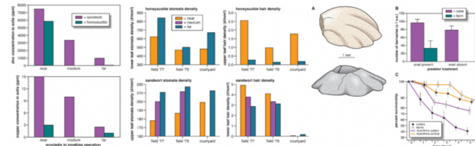
Variation, mutations and independent assortment



Giuseppe Bianchi G, et al (1994) [Point mutations in adducin genes in blood pressure variation](). Proc. Nat. Acad. Sci. USA 91: 3999-4003.

- (16.1 sandworts & barnacles)

Variation caused by the environment



Nicholas Caiazza, Quinn JA (1980) [Leaf morphology in A.patula & L.japonica along pollution gradient.]() Bulletin Torrey Bot. Club 107(1): 9-18.

Why do we need annual vaccines? (Non-Mendelian genetics)

- (16.3 flu vaccines)

Random mix of chromosomes leads to rapid (non-mendelian) evolution

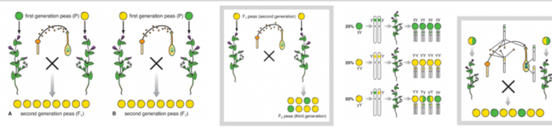
Lu J, Gu J, Li K, et al. (2020) [COVID-19 Outbreak with Air Conditioning in Restaurant. Emerging Infectious Diseases](). 26:1628-1631.

Organisms—Reproduction and Cell Division: How cells divide and pass DNA information to the newly produced cells.

How can traits disappear and reappear in a later generation? (Mendelian genetics)

- (3.1 Mendel's peas)

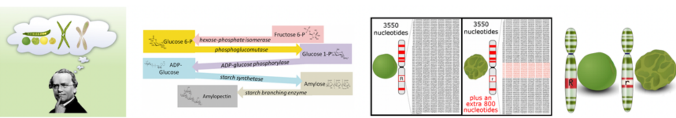
Traits discovered by Gregor Mendel in pea plants



Gregor Mendel. 1866. [Versuche über Plflanzenhybriden. Verhand- lungen des naturforschenden](), Bd. IV für Jahr 1865, Abhandlungen, 3–47.

- ([Evo-Ed.com]() SBE1 gene)

Gene for traits discovered by Mendel in pea plants

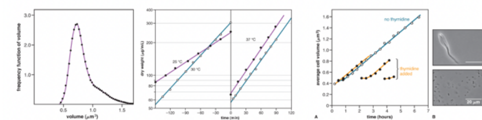


Madan K. Bhattacharyya, Alison M. Smith, et al (1990) [The wrinkled-seed of Mendel is caused by a transposon-like insertion](). Cell, 60, 115-122

How do prokaryotes communicate their identity to the next generation? (Cell Division)

- (3.3 *E.coli* cell division)

Prokaryotes use cellular fission to produce daughter cells



Kubitschek HE. (1971) [Control of cell growth in bacteria: experiments with thymine starvation](). J Bacteriol 105(2):472-476.

Do eukaryotes produce new cells the same way as bacteria? (Mitosis & Meiosis)

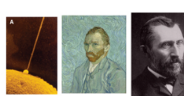
- (3.4 kangaroo mitosis)

Eukaryotic DNA must be partitioned in daughter cells

name	description	duration (hours)
G_1	growth and normal cellular functions	10
S	synthesis of DNA	8
G_2	growth and normal cellular functions	4
mitosis	separation of chromosomes	2

- (3.5 meiosis & ELSI)

Meiosis produces unique chromosomes

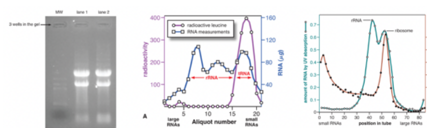


Cells—Central Dogma: How cells process molecular information from DNA to RNA to protein.

How does DNA communicate information to the cell? (Transcription & Translation)

- (2.1 RNA types)

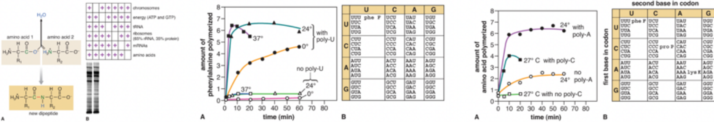
Gene activity controls timing &level of RNA transcription



Margaret Chipchase, Birnstiel ML. Synthesis of transfer RNA by isolated nuclei. Proc Natl Acad Sci USA. 49(5):692-699, 1963.

- (2.3 RNA translation)

How do cells make proteins from RNA?



Nirenberg MW, Leder P. RNA codewords and protein synthesis: effect of binding of SRNA to ribosomes. Science 145(3639):1399-1407, 1964.

- (2.4 RNA insulin & NCBI)

What is a gene, can cells choose information?? *NCBI Tutorial*

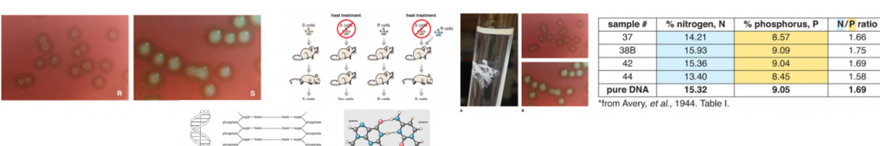
Gerstein MB, Bruce C, Rozowsky JS, et al. What is a gene, post-ENCODE? History and updated definition. Genome Res 17(6):669-681, 2007.

Molecules—Heritable Information: Identifying the heritable substance.

What is biological Information? (Heritable Material)

- (1.1, 1.2 Griffith & Avery)

What is the heritable material?



sample #	% nitrogen, N	% phosphorus, P	N/P ratio
37	14.21	8.57	1.66
38B	15.93	9.09	1.75
42	15.36	9.04	1.69
44	13.40	8.45	1.58
pure DNA	15.32	9.05	1.69

*from Avery, *et al.*, 1944. Table I.

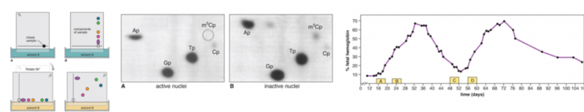
- (1.4 DNA structure)

How does DNA's shape affect its function?

Watson JD, Crick FH. nucleic acids. Wilkins MH, et al. Molecular ... Franklin RE, Gosling RG. Molecular configuration. Nature 171:737, 1953.

- (1.5 HELLO epigenetics)

Is all genetic information encoded in the DNA?



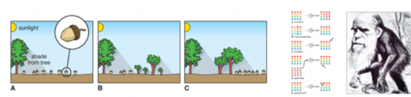
DeSimone J, Heller P, Hall L, et al. 5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons. PNAS 79(14):4428-4431, 1982.

Evolution of Life, Earth and Humans: How chemicals, cells and humans developed over time.

What is evolution? (evolution & origin of life)

- (4.1, ELSI 4.1 evolution)

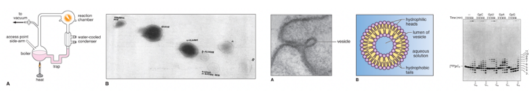
The origin of life by natural processes continues to evolve.



Charles Darwin. On the Origin of Species, 1859, Darwin Online : https://darwin-online.org.uk/converted/pdf/1860_Origin_F376.pdf

- (4.2 Stanley Miller, origin of life)

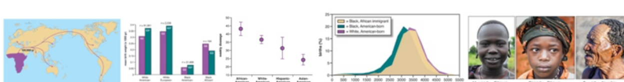
Could abiotic chemicals form biological molecules?



Miller SL. A production of amino acids under possible primitive earth conditions. Science 117(3046):528-529, 1953.

- (6.5 Human Evolution)

What do our genomes tell us about human evolution?



Collins JW Jr, Wu SY, David RJ Differing intergenerational birth weights in Illinois. Am J Epidemiol. Feb 1;155(3):210-6. 2002

THE TEXTBOOK

What is so insanely great about this textbook?

Drs. Malcolm Campbell, Chris Paradise and Laurie Heyer wrote an extraordinary textbook for learning biology. Over the past 50 years, research in biology has become more quantitative and interdisciplinary, relying more heavily on other sciences. To understand large ecosystems, or to make sense of massive data from human and other genomes, today's biologists must be able to use modern math, statistics, computation, and tech tools.

Yet biology instruction and traditional textbooks have also not kept pace with modern biology nor current research into how people learn. Studies on *learning* reveal that students learn best if they are actively engaged working both individually and in groups together constructing their own knowledge.

The textbook *Integrating Concepts in Biology* takes advantage of these insights and enables you to better achieve your full learning potential by directly involving you in your own learning[1].

You will be asked to construct your own knowledge by analyzing and interpreting published data. As you gain knowledge, you will find you can learn more and retain new information more easily. Our classroom discussions will help you learn how to read text and scientific figures. You will be able to learn major concepts by reading about several examples in more depth. The textbook readings, online homework and in class discussions will guide you in interpretation, analysis and will help you build your new skills and knowledge.

The textbook does *five* things that experts[2] have always said "should be done" in biology textbooks:

1. **Biology data**: You are presented both historical and modern published research data used to answer biological questions. You practice interpreting that data, making sense of it, just as scientists do.

2. **Hierarchy/Scale**: BIG biology (organismal) and little biology (molecular/cellular) is addressed together, integrated. The textbook integrates information across the biological size/hierarchy and scale.

3. **Big Ideas**: The text focuses on big ideas, you will focus on these big ideas of biology.
- 1) INFORMATION: Living system's mechanisms to store, retrieve, and transmit *information*.
- 2) EVOLUTION: The diversity and unity of life can be explained by the process of *evolution*.
- 3) CELLS: Cells are a fundamental structural and functional unit of life.

4. **Math**: Mathematics is used as an important tool. Self-contained Bio-Math Explorations (BMEs) help you understand how math is applied to answer biological questions.

5. **HPS ethics**: Finally, the text raises your awareness about ELSI (ethical, legal, and social implications) and you engage with case studies of real-world implications of what you are learning.

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[1] Barsoum M, Sellers PJ, Campbell AM, Heyer LJ, Paradise CJ. Implementing recommendations for introductory biology by writing a new textbook. CBE Life Sci Educ. 2013;12:106–116.

[2] Vision and Change By the American Association for the Advancement of Science, A New Biology for the 21st Century By the National Research Council, Trivializing Science Education By Bruce Alberts, "Computing Has Changed Biology: Biology Education Must Catch Up" By Pavel Pevzner and Ron Shamir, "Revising the AP Biology Curriculum" By William B. Wood, "Mathematical Biology Education: Beyond Calculus" By Raina Robeva and Reinhard Laubenbacher, "Student Use of Out of Class Study Groups in an Introductory Undergraduate Biology Course" By Stephen M. Rybczynski and Elisabeth E. Schussler, "A Study Assessing the Potential of Negative Effects in Interdisciplinary Math Biology Instruction" By Andreas Madlung, Martina Bremer, Edward Himelblau, and Alexa Tullis, "Effects of Collaborative Group Composition and Inquiry Instruction on Reasoning Gains and Achievement in Undergraduate Biology" By Jamie Lee Jensen and Anton Lawson, Connecting Learning to Teaching By John Girash

Integrating Concepts in Biology
By A. Malcolm Campbell, Laurie J. Heyer, and Christopher J. Paradise

Table of Contents

In the entire textbook, each of the five units focuses on one of the Big Ideas of biology. Each chapter in a unit focuses on a particular level of the biological hierarchy. We will focus on two Big Ideas (two units).

Big Idea 1: Information
Living systems have multiple mechanisms to store, retrieve, and transmit information. Main ideas include:
1. Heritable information provides for continuity of life.
2. Imperfect information transfer produces variation.

Big Idea 2: Evolution
The diversity and unity of life can be explained by the process of evolution. Main ideas include:
1. The origin of living systems occurred by natural processes, and life continues to evolve.
2. Natural selection is a mechanism of evolution that accounts for adaptation.

you are here		Big Ideas of biology				
		Information	Evolution	Cells	Homeostasis	Emergent Properties
levels of the biological hierarchy	molecules	1	4	7	10	13
	cells	2	5	8	11	14
	organisms I	3	6	9	12	15
	organisms II	16	19	22	28	25
	populations	17	20	23	29	26
	ecological systems	18	21	24	30	27

Chapter 18: Discusses commonalities in communication within and between species, how information is used by organisms to find and exploit other species. Case studies include: (i) mole crickets versus parasites, (ii) frog choruses and predators (opossums and bats), and (iii) where corals decide to grow.

Chapter 17: Discusses language and information transfer between organisms. How animals communicate and find each other through signals, using light or sound. Case study stories include: (i) fireflies, with light signals, (ii) a story about bird vocalizations, (iii) meerkats vs. mongooses (iv) how do plants communicate?

Chapter 16 & 3: In these chapters, you will learn how to predict patterns of inheritance and how organisms passed their genetic information to future generations. How prokaryotes (E. coli) reproduce, how eukaryotes use mitosis and meiosis. The laws discovered by Gregor Mendel and viral mechanisms.

Chapter 2: In this chapter, you will follow the path of researchers who made many ground-breaking discoveries about how cells produce proteins, processes that were nicknamed "central dogma" (transcription and translation) that answer "how does DNA communicate information to the cell?"

Chapter 1: You will explore and interpret the original data from experiments that led to our current understanding of DNA as heritable information. Case studies presented are: (i) Griffith and Pseudomonas bacteria story, then (ii) Oswald Avery's data and story, (iii) the Watson, Crick story with Franklin and Wilkins; and a discussion of how methylation of DNA sequences reduces expression.

Chapter 4-6: You will analyze data that illuminate the origin of eukaryotic cells from prokaryotic ancestors, the mechanisms of evolution (natural selection, mutation, gene flow, genetic drift), as well as evidence on how life on earth evolved (NASA, Stanley Miller experiment, RNA World hypothesis). And you will evaluate data regarding complexity of human biology in areas of medicine and evolution.

THE LECTURE
ASSIGNMENT SCHEDULE

Researchers have found increased structure and active learning increase everyone's ability to learn in introductory biology courses[3]. In addition, every student in our course really does want to slowly carefully read the textbook, learn new information and enjoy mastering topics in biology. Given we believe the textbook we are using is outstanding, we are structuring short readings, with integrating questions in the course, so you more carefully read each section and reflect upon it. A quiz or exercise based on the reading may be given each lecture. These quizzes/exercises are designed to help you assess your own learning before and between exams. They provide you with regular feedback as to how well you are mastering each topic.

ATTENDANCE AND PARTICIPATION: It is essential that you not only come to class but also actively participate in order to construct your own knowledge. While attendance is being "present", participation includes reading and preparing well for class, answering questions verbally, and via clicker questions. Active participation includes, the following behaviors:
1. *Bringing forth new ideas, information, or perspectives to academic conversations*
2. *Discussing your readings and reflections with instructors and peers*
3. *Meeting with the instructors to discuss your interests, assignments, or project*
4. *Participating in small group discussions and activities*
5. *Assuming responsibility for personal behavior and learning*

While working on group projects, students should be mindful, all participants should exercise:
- *Respect for themselves, each other*
- *Openness and a positive attitude toward new ideas and other's ideas*
- *Flexibility and tolerance of ambiguity*
- *Good communications amongst themselves.*

EXAMS: There will be two midterm exams and a final exam, each may be comprehensive of all prior material. Midterm exams may be traditional multiple-choice format, or may be essay-style Answers to open-book & take-home exams must also be submitted online to http://turnitin.com/.

Assignments (pts)**:**

Week	Assignment(s)	@Lecture	%
(*all*)	Attendance, Participation, Homework, Quizzes	X	**20**
5	**Exam I**	**X**	**25**
10	**Exam II**	**X**	**25**
15	**Final Exam**	**X**	**30**

Total = 100% of lecture grade

Tardis Pass - - One-Time Time Travel RE-DO permit, improve work via revision opportunity.

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[3] Haak, D., J. HilleRisLambers, E. Pitre, and S. Freeman. 2011. Increased structure and active learning reduce the achievement gap in introductory biology. *Science* 332:1213-1216. Freeman, S., D. Haak, and M.P. Wenderoth. 2011. Increased Course Structure Reduces Fail Rates in Biology. *CBE Life Science Education* 10 (2):175-186

HYBRID EDITION

LB-144: CELL & ORGANISMAL BIOLOGY (LABORATORY)

LAB COORDINATOR

Douglas B. Luckie, Ph.D., Associate Professor, Lyman Briggs College & Dept. Physiology

LAB MANUAL

found inside "LB-144 Course Pack," (Luckie) from MSU Library Services via local bookstore

COURSE WEBSITE http://ctools.msu.edu/144

RESEARCH TEAM RATIONALE

Student groups are intended to be research & learning teams. Work with other students to study and discuss biology topics in lecture, as well as share your ideas and research predictions in lab. Teams are better learning environments but also, they are REAL LIFE. While scientists do some things on their own, they more often work in groups to solve problems because a well-functioning team is the most efficient way to work. Working in the same group in both laboratory and lecture will allow you to become more familiar with each other so you will feel comfortable enough to discuss your biology questions. Although it is easier for an instructor to run a class or lab without group work, numerous research studies have shown that working in groups and discussing science with your peers can increase your learning ***considerably*** (although you have to strive to be a “cooperative” group). By pooling your knowledge, members of your group will get “stuck” less often be able to progress far beyond what any individual in the group could do alone.

Week	Before Lab Meeting	During Laboratory Meeting Activities & Assignments DUE
1	*ONLINE LAB*	*Talking to Strangers* Film, Quiz, Honey Guide paper
2	View "IDEO" Film	Film Quiz & Debrief, Writing INTROS, Form Groups
3	View "Islands" Film	Film Quiz, 4-slide Proposal Talk & movie, Grading TITLES
4	Group Contract	***2¶-Draft due***, Preparing for LA and Prof Thesis Interviews: Q&A
5	*GEA1* on Catme.org	**LA Interviews begin** (during & outside lab time, groups of 4, 60m)
6		**LA Interviews (cont.)** Writing RESULTS & FIGURES
7		***Half-Draft due*** (2¶+ Results/Figs paper), Grading FIGURES
8	*GEA2* on Catme.org	**PCR** & **Prof Interviews begin** (during lab, in groups of 2, 60min)
9-12		Gene research (PCR, gels, Primers, BLAST =Molecular Teams)
13		***Final film*** and/or ***Final paper (DRAFT1) due***
14-15	*GEA3* on Catme.org	**Prof Interviews completed** (during lab, in groups of 2, 60min)

THE LABORATORY

You will need the Laboratory Manual resources provided in the Course Pack. Review the lab guide materials required for each week during the semester. This semester, you will design and pursue one experiment all semester long. You will find an interesting animal behavior related to communication that has been studied and published in the literature (like a mating display) and attempt to document it when observing animals on locally (like squirrels & humans). 4.0-seeking students will also connect the behavior to a gene. Your group will capture your observations with still photographs and digital video from your smartphones. Ultimately, you'll generate a short 5minute documentary film showing the results of your research and write a formal research manuscript. Each week, you will examine and practice the methods of a scientist in performing your research. This approach is aimed at mentoring you, so you master the ability to think and work like a serious scientist.

While working on group projects, you should be mindful of other students in your group; therefore, it is important for all participants to exercise:

- Respect for themselves, each other
- Openness and a positive attitude toward new ideas and other's ideas
- Flexibility and tolerance of ambiguity
- Good communications amongst themselves

ASSIGNMENT SCHEDULE & VALUES

Speaking (value)	*Writing (value)*	*Discussing/Demonstrating*
Proposal talk & movie= 10%	Proposal 2¶-Paper= 10%	LA Thesis interview= 10%
	Half-Draft Paper= 20%	Prof Thesis interview= 20%
	Final Paper/Film=30%	

Week	**Assignment(s) Due**	**Value (%)**
3	**Proposal Talk & movie**	**10**
4	**Proposal *2¶-Paper***	**10**
5	*LA Thesis Interview* (individual score, group format)	**10**
7	***Half-Draft Paper***	**20**
7-15	*Prof Thesis Interview* (individual score, pair format)	**20**
12	Final **Paper** -or- (Film option)	**30**
	Total	**= 100% of lab grade**

Tardis Pass **-**- One-Time Time Travel RE-DO permit, improve work via revision opportunity.

The "Honors Option" (optional)

*Note: The Honors Option for LB144 this semester is presenting your group's research findings as a talk at the UURAF during the Spring Semester. This is required to be an individual assignment (not done as a group) and a talk (not a poster) if you seek individual credit for an Honors Option. Be aware the UURAF application deadline is often in January.

MSU & LBC INFORMATION AND POLICIES

Face Coverings:
Face coverings must be worn by anyone who is not vaccinated versus the SARS-CoV-2 coronavirus (all faculty, staff, students, vendors, and visitors) while participating in MSU-related or MSU-sponsored activities. If you have a medical condition that may prevent you from safely wearing a face covering, you should contact MSU's Resource Center for Persons with Disabilities to begin the accommodation process.

Face coverings should (a) be non-medical grade to maintain supplies for health care use, (b) fit snugly against the side of your face, (c) cover your nose and mouth, (d) be secured with ties or ear loops, and (e) allow for breathing without restriction. Cloth face coverings should only be worn for one day at a time, and they must be properly hand washed or laundered before subsequent use. Face coverings may vary (for example, disposable non-medical face coverings or neck gaiters are acceptable).

Absence due to illness: Students who need to quarantine themselves, have been sick with COVID-19 symptoms, tested positive for COVID-19, or have been potentially exposed to someone with COVID-19 must follow CDC guidance to self-isolate or stay home. Illness or self-isolation will not harm performance or put one at a disadvantage in the class.

Technical Assistance
If you need technical assistance at any time during the course or to report a problem you can:

- Visit the Distance Learning Services Support Site
- Visit the Desire2Learn Help Site (http://help.d2l.msu.edu/)
- Or call Distance Learning Services: (800) 500-1554 or (517) 355-2345

Mental Health Resources
College students often experience issues that may interfere with academic success such as academic stress, If you or a friend is struggling, we strongly encourage you to seek support. Helpful, effective resources are available on campus, and most are free of charge.

- Drop by Counseling & Psychiatric Services (CAPS) main location (3rd floor of Olin Health Center) for a same-day mental health screening.
- Visit https://caps.msu.edu for online health assessments, hours, and additional CAPS services.
- Call CAPS at **(517) 355-8270** any time, day or night.
- 24-Hour MSU Sexual Assault Crisis Line **(517) 372-6666** or visit https://centerforsurvivors.msu.edu/

Resource Persons with Disabilities (RCPD)

- To make an appointment with a specialist, contact: (517) 353-9642
  Or TTY: (517) 355-1293
- Web site for RCPD: http://MYProfile.rcpd.msu.edu

Inform Your Instructor of Any Accommodations Needed

- From the Resource Center for Persons with Disabilities (RCPD): Once your eligibility for an accommodation has been determined, you will be issued a Verified Individual Services Accommodation ("VISA") form. Please present this form to me at the start of the term and/or two weeks prior to the accommodation date (test, project, etc.).

LBC Student Success and Advising Team

LBC advisors work to educate, coach, and support students in our College. For more information about the Student Success and Advising team visit: https://lbc.msu.edu/advising/index1.html

To make a zoom or phone appointment with an advisor visit: https://lbc.msu.edu/advising/advising-appointments.html

To review LBC Academic Policies, including LBC's Academic Grievance Policy, visit: https://lbc.msu.edu/advising/academic-policies.html

Related Policies:
Institutional Data Policy:
https://tech.msu.edu/about/guidelines-policies/msu-institutional-data-policy/
Student Privacy Guidelines and Notification of Rights under FERPA
https://reg.msu.edu/ROInfo/Notices/PrivacyGuidelines.aspx

Commitment to Integrity: Academic Honesty
Article 2.3.3 of the Academic Freedom Report states that "The student shares with the faculty the responsibility for maintaining the integrity of scholarship, grades, and professional standards." In addition, the (insert name of unit offering course) adheres to the policies on academic honesty as specified in General Student Regulations 1.0, Protection of Scholarship and Grades; the all-University Policy on Integrity of Scholarship and Grades; and Ordinance 17.00, Examinations. (See Spartan Life: Student Handbook and Resource Guide and/or the MSU Web site: www.msu.edu.)

Therefore, unless authorized by your instructor, you are expected to complete all course assignments, including homework, lab work, quizzes, tests and exams, without assistance from any source. You are expected to develop original work for this course; therefore, you may not submit course work you completed for another course to satisfy the requirements for this course. Also, you are not authorized to use answers provided by Chegg.com or CourseHero.com or similar "cheat" web sites to complete any course work in this course. Students who violate MSU academic integrity rules may receive a penalty grade, including a failing grade on the assignment or in the course. Contact your instructor if you are unsure about the appropriateness of your course work. (See also the Academic Integrity webpage.)

LINKS TO UNIVERSITY POLICIES

- **Spartan Code of Honor**
- **Academic Integrity**
- **RCPD Disability Accommodations Statement**
- **Mental Health**
- **Tolerance and civility**
- **Religious Observance Policy**
- **Student Athletes**
- **MSU Final Exam Policy**

Owner's Manual
(with lots of ideas and text stolen from great authors, Drs. Alice Dreger and Tanya Noel)

Why is this an "owner's manual" instead of a syllabus?

Most syllabi contain only class schedule information. By contrast, this is more like an "owner's manual" like the sort that comes with a new car. If you read and use this manual, you will understand how this course works, and you will be able to keep the course running smoothly, and do the regular maintenance required to avoid breakdowns. Of course, this course isn't a car. It's more like a bus tour. I believe that a university course is in its essence not a number, and not a topic, but a group of people who share a common goal of learning about some particular thing. In this sense, a course is like a bus tour, a tour to a place which is unfamiliar to most of us. As the teacher, I am the bus driver and chief tour guide. Each member of the course starts off at "home" intellectually and emotionally and comes to the bus station which is the classroom. We agree to "take the tour" together, to get on the bus and travel together for the length of the course even though many of us may never have met before. Together we visit a number of different "places."

So why is this "owner's manual" so long?

I've discovered that the more information I give students, the more comfortable and in control they feel, and the better they learn. This packet contains lots of information. Besides telling you about the mechanics of the course, this packet tells you a lot about my teaching style. I used to provide my students with a separate "statement of teaching philosophy." It now occurs to me it is weird to separate that teaching philosophy from my teaching materials. So now my philosophy is embedded throughout this packet. My teaching style, methods, and philosophy change over time, thanks to students who tell me what works and what doesn't work. I'm counting on you to give me lots of feedback about what is working for you and what is not, and most importantly why. It is very important to me to do a good job for you. In addition to the course learning objectives provided earlier, be aware this course aligns with the following MSU Undergraduate Learning Goals:

Analytical Thinking
A successful student uses ways of knowing from mathematics, natural sciences, social sciences, humanities, and arts to access information and critically analyzes complex material in order to evaluate evidence, construct reasoned arguments, and communicate inferences and conclusions.

- *Acquires, analyzes, and evaluates information from multiple sources.*
- *Synthesizes and applies the information within and across disciplines.*
- *Identifies and applies, as appropriate, quantitative methods for defining and responding to problems.*
- *Identifies the credibility, use and misuse of scientific, humanistic and artistic methods.*

Effective Communication
A successful student uses a variety of media to communicate effectively with diverse audiences.

- *Identifies how contexts affect communication strategies and practices.*
- *Engages in effective communication practices in a variety of situations and with a variety of media.*

Integrated Reasoning
A successful student integrates discipline-based knowledge to make informed decisions that reflect humane social, ethical, and aesthetic values.

- *Critically applies liberal arts knowledge in disciplinary contexts and disciplinary knowledge in liberal arts contexts.*
- *Uses a variety of inquiry strategies incorporating multiple views to make value judgments, solve problems, answer questions, and generate new understandings.*

How does this course work in terms of the day-to-day?

We will meet two times a week for the lecture class and our meetings will consist of discussions of the readings and activities related to the topics we are investigating. Do the readings assigned for the day **before** you come to class and spend enough time thinking about the readings before class. You should come to class ready to summarize the readings and to ask and answer questions about them. Homework and quizzes will often be given on the readings.

Always give yourself plenty of time to do your work, and feel free to contact me whenever you need help or clarification. I like teaching and not only do I feel good when you learn, often when you

learn something new, I learn, too.

Generally, we will stick very closely to the attached schedule, however, the point of this class is for you to learn, so if we need to change our scheduled plans to achieve that goal, we will do so. If you feel that you need things to be done somewhat differently in class in order for you to learn better, please let me know and I will work to adjust our schedule or classroom dynamics so that we can maximize learning.

So what's my feeling about teaching?

I love it! And I think it shows – my students have voted me "honorary member of the graduating class of Lyman Briggs" ("teacher of the year") about five times in the last fifteen years, I was given the Teacher-Scholar Award of MSU, and most recently the 2015 MSU Alumni Club of Mid-Michigan Quality in Undergraduate Teaching Award (nominated by MSU faculty and alumni for teaching) and the 2017 Outstanding Faculty Award by the ASMSU Senior Class Council (nominated by MSU graduating seniors for teaching). If you hear that I am tough, I am, but that's because I care about your learning. If I didn't care about your learning, I would have stayed at Stanford University.

I am delighted to have recruited amazing LAs to help you do well in the course. You will find that our LAs share my love of teaching, of biology and dedication to helping you learn. But they are tough too because they want you to learn, lots. They are trained to answer your questions with responses in the form of guiding questions. Why? because it helps you learn and *remember*, and they know your next class (and career) will be far more difficult and demanding than this course, you know this too.

What else besides being in class will be required of you?

Note that this course uses a wider range of assignments than just several exams. This spreads out risk and stress so it's lower level, day to day, and allows you to assess your own learning with lower-stake quizzes to avoid any surprises when facing the bigger exams. Grades are pretty simple, like getting an "A" or "B+" or "C" written at the top of each assignment– and you can always check your grade on the D2L gradebook – but be sure to keep your own spreadsheet and alert me if my gradesheet has an error.

- *Quizzes on readings:* I will frequently give short quizzes on a day's assigned reading at the beginning of the class meeting. These quizzes accomplish two things: (1) reward you for keeping up-to-date on the readings; (2) reward you for spending enough time on the readings to really understand them. If you read carefully, you should have little problem with the quizzes. If you have a lot of trouble with short, fast quizzes, remember there are lots of bonus options in this class you can use as substitutions. If you miss a quiz because you are late or absent, you will receive a "0". These cannot be made up.

A note on grades & FERPA:

To support blind-grading we will often request that you not list your actual name but just provide your PID. Privacy, as required by MSU FERPA regulation, will be maintained by utilizing a code that is NOT your real A-PID, so we'll call it your B-PID. Your B-PID will be listed on D2L in your personal gradebook.

Backstory: In recent years universities have become very afraid of getting in trouble for breaking the law called FERPA (Family Educational Rights and Privacy Act). The law was created back in 1974 to protect the privacy of students and their grades. In response to it all universities created student ID numbers so instead of placing a grade next to a person's name, instructors could place it next to a student number to maintain privacy. Many universities chose to use a student's social security number to also be their student number. When identity theft became a big problem, universities then changed all their

student ID numbers from social security to become some number randomly generated in house. In recent years now the student ID number itself has become protected. In fact, while other people are permitted to know your name, and even say it aloud and post it publicly, the student ID number is super protected. Thus instead of using your officially MSU-issued A-PID, in this course MSU requires that we issue a new temporary student ID. We will call these the B-PID, since they are for "who you be" and it's for blind grading.

Professors can use grades in two ways: they can use grades to "sort" students into "A" students, "B" students, etc.; or they can use grades as learning incentives and rewards. Unfortunately the sorting system generally sorts according to "talents" students either have or don't have before they ever reach a particular classroom, e.g., the talent of being able to memorize and recall a lot of things. I would rather use grades to encourage students to develop their skills, to expand their minds and interests. While students are often only familiar with positive curving (sometime called a mother's curve) a number of university classes use an actual curve that raises or lowers the grading scale with the goals to only permits a few students (like just 10 in a class of 100) to earn a 4.0 and then only a few (perhaps 20) are permitted to have a 3.5 etc. Even if everyone in the class got above a 90% on an exam the grade scale would shift up until only the prescribed number of students got a 4.0 grade. This is a real "curve" and, I will never grade on a curve like this. Our grading scale with stay exactly as stated in the syllabus and each student will get whatever grade she or he has earned by the end of the semester. Nothing would make me happier than if everyone worked hard and learned a lot and got 4.0's. I would feel that we had achieved something great if everyone got a 4.0.

Table 1- University-level grading system: The table below describes the relationships between grades, percent, and performance in the University-level grading system used in our lab and lecture courses. The first column describes the letter/number grade. The second column describes the percentage associated with that grade. The third column describes the performance-level required. Remember, if at any point you feel confused or distressed about your grades, carefully review the syllabus and talk to me.

Letter Grade	***Percentage***	***Performance***
A (4.0)	90 to 100%	*Outstanding Work*- A "4.0" is Outstanding. It literally stands out. It has the characteristics described for 3.0 and 3.5-level elements but in addition, the work by itself impressed with how much & well it was done. The student taught Prof something original.
B+ (3.5)	85 to 89.9%	*Most Excellent Work* - A "3.5" is Most Excellent. Every detail of the work was done extremely well and they found additional papers and evidence beyond what they were told.
B (3.0)	80 to 84.9%	*Excellent Work* - A "3.0" score is considered Excellent. It is impressive work, top of the class, and the work was done extremely well but nothing beyond what was expected.
C+ (2.5)	75 to 79.9%	*Pretty Good Work*- A "2.5" is Pretty Good, the student did the minimum work required and did a pretty good job, this is expected at the university level and near average for the class.
C (2.0)	70 to 74.9%	*Average Work* - A "2.0" is average, the student did the minimum work required.
D+ (1.5)	65 to 69.9%	*Below Average Work* - the student did less than minimum work required.
D (1.0)	60 to 64.9%	*Poor Work*- the student did less than minimum work required and of poor quality.
F (0.0)	0 to 59.9%	*Failing Work*- the student did far less than minimum work required and very poor quality.

Course Structure

This course will use a public website and online tools like Turnitin, CATME, Desire2Learn, and Top Hat. The course website may include online lessons, course materials, and additional resources. Activities may consist of readings, discussion forums, email, journaling, wikis, and other online activities. You will need your MSU NetID to login to the course to access the grades on *D2L* (*http://d2l.msu.edu*).

Definitions, terms, transparency

Admission: I believe caffeine and sugar increase attention and learning but have no empirical data to support this, except for eating donuts, that is documented to work, but just for 15 minutes post-eating. I like the drink called the Cortado (it's coffee, like a tiny latte) but particularly enjoy the moment I pour cane sugar out of the brown paper packet on top of the frothed milk and watch it sink into the drink. When you come to office hours, unless there's a rush, I'll likely offer you an espresso (LIVE or virtually).

Attendance: Student learning is impacted by many things, yet education research has robustly shown it is significantly impacted by these three things: class size, teacher quality and attendance. You are, of course, permitted to skip any class meeting you wish but often a single clicker point is made available to you, to encourage attendance since it correlates with learning. Attendance at the meeting of a class will be defined as being physically present in the room for the full time period of the class meeting. Thus be present, in your seat with you notebook open and pen in hand, at the very beginning when the clock in room strikes the hour and class begins, still there during/throughout the entire duration of the class, as well as at the very end of the official time period (feel free to come and go to visit the restroom, just not off vacationing elsewhere). It's only fair to treat students who arrive late exactly the same as those who depart early. We will often reward students for attendance by using technology to record your presence. If you fail at using your device to click-in for attendance at the beginning middle or end of class, due to whatever reason, be aware we do not micromanage the attendance data (no appeals). Making the choice to schedule another course that has a start or finish time that is proximal or even overlaps with this class is, of course, your choice and entirely acceptable. Yet this will not change the definition of attendance or waive it. University students are adults and literally everything in a course is optional, yet if you want points, in this case for attendance (and more importantly to learn) you have to be there.

Belong: Lyman Briggs College is dedicated to promoting inclusion and fostering diversity. Let's make our classroom comfortable and welcoming for everybody. Let's strive to treat everyone with respect, civility, and empathy and rather than avoid new things to learn from others about different beliefs, practices, and lives. You are all super wonderful smart people and all belong here.

Blind grading: When a computer scores a scantron bubble sheet from a multiple choice exam, it is objective, it doesn't have a pre-conception as to which students are smart, or are nice to it, so it treats everyone the same and just rewards correct answers. Unfortunately, human graders are less objective. LAs, GTAs, and Profs, are all unable to be perfectly objective when they have already had interactions with the person whose work they are grading. While they try hard to be so, education research shows that even knowing what the person's name is will impact the grader and grade (even if they never met the person). Thus imagine if they know the person reasonably well. If they have read prior papers, knew the person's prior grades, or had a number of positive (or negative) conversations with them. Wow, that will cause major problems when trying to be objective while grading, even for the best teacher ever, unless the grader is blind to the identity of the author. Professional journals and grant review panels use single blind or double blind systems to avoid subjective evaluation. We will use this in our class too.

Participation: It turns out participation is different than attendance. It refers to a student who is actively working to learn the materials discussed in the course. Students who are active participants do not merely talk during class but also prepare in advance for class. This means carefully completing the readings, taking notes on them (best for learning, do this by handwritten notes on paper) and preparing for the upcoming class meeting by reviewing notes and highlighting any questions you thought of while preparing for class. To reward this behavior, which enhances learning, often there will be a pop quiz or problem or writing exercise during class which is scored. Also there are clicker questions during lecture and you earn a point each time you chose a correct answer. You only need to get *half* of all clicker points to earn a perfect 100% score for participation. And, if you go below that level, you still get a 50% score if you tried. If you prepare for class, you'll get good grades, and if you don't, you get at least get some credit for trying. This helps increase the number of people that ultimately decide they need to study the material prior to class and as a result also learn more when discussing the material again in class. If you prepare, class is fun and interesting. If you don't, it can become confusing and frustrating, as it feels like everyone else seems to know all the answers while you don't even understand the questions.

Random calling in lecture: How often have you been in a big lecture class that has maybe 8 students who are the only people who ever are called upon to answer the professor's questions in lecture? The other 100+ students throughout the entire semester will generally never speak aloud during lecture. After a while you get used to it. Everyone knows that "those students" answer the questions, so we don't have to, cool. Yet, deep down you also know, while it's comfortable to never have to answer a question, it likely reduces your learning, heck some folks fall asleep. My wife tells a story about a small class where the Professor always asked these incredibly difficult questions that nobody ever even understood. Then one day, near the end of the semester, she did the reading prior to class and during class realized that ever single question the instructor asked was directly out of the reading. She was embarrassed because she realized they must know nobody does the reading, given no student ever understood the questions he asked even though they were right out of the first pages of each reading. Because our goal in this class is learning we will use random calling in lecture to help *everyone* increase their learning and gain skills at communication/public speaking.

---"Tips from Tanya": Some points for students about technology in the classroom ---
Author: Dr. Tanya Noel

Almost everyone has a smartphone, laptop, tablet, or combination of these devices with them during their waking hours (and beyond, in some cases). There is huge potential for distraction using these devices – which is fine if you're waiting in a long, boring line or on the bus, but can be problematic in the classroom. Be aware of:

- There have been studies that have shown **"multi-tasking" in class is detrimental to learning**. (Actually, the evidence overwhelmingly suggests humans can't really multi-task … or, at least, can't multi-task well!) If you're trying to go back and forth between course-related stuff and other websites (or assignments for other courses, etc.), this will affect how well you're learning/working.

- **Notifications (e.g., beeps/vibrations for new emails, text messages, etc.) are highly distracting**, and feed into "reward systems" in the brain that can reinforce behaviors like frequently checking your phone, Facebook, etc. (You know that uncomfortable feeling that makes you check your phone/email? Your brain gets a dopamine hit when you give into that urge … and makes it more likely to continue the behavior leading to the reward.) **Consider turning off these notifications**, at least during class and other times when you want to be able to focus uninterrupted. (Some people

have found turning off notifications altogether has helped them not only focus, but reduced their stress levels!)

- **Note-taking on computers (vs. by hand) is associated with lower-quality learning/test scores**. Results from some recent studies support the idea that writing notes by hand on paper is superior to taking notes on the computer. There are a number of hypotheses about this, but many experts agree that taking notes by hand involves more thinking about what's important and worth writing down (as you can't transcribe every word spoken by the professor). On the computer, it is tempting to try to record everything verbatim, with the brain not processing much of the information.

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Chapter Checklists for each lecture

I strongly suggest attaching each Checklist sheet for the current week to your "work wall" (e.g. bulletin board) in your room where you can see it at a glance and literally check-off items as you complete them.

Before first day of class:

Obtain supplies for course: online textbook, printed course pack, online homework system. *See course website for more details (http://ctools.msu.edu/144)

1. _______ **Buy** ($36) our online **Integrating Concepts in Biology (ICB) textbook**. Do not buy an expensive $259 Biology textbook! Please just buy this inexpensive one. Note: this textbook is custom-assembled with just the chapters for this class, so unless you want the full textbook, buy the LB144 Luckie version of the ICB textbook (http://store.trunity.com/ just search for "Luckie").

2. _______ **Buy** ($26) access to **TopHat online homework** & **clicker system.** Do not spend $100+ for an online homework system like Mastering Chemistry/Biology and then also buy a $50 clicker you might break or lose. Please just buy the TopHat online homework system for the semester, which also permits you to use your phone/tablet/laptop as a clicker. Click the TopHat.com link on course website.

3. _______ **Buy** ($5) traditional paper **Lecture Notebook.** Can be spiral bound or 3-ring bound as long as there is real paper that you'll write on lots (mostly for taking notes when doing the readings prior to lecture). Be sure to write all notes by hand, it greatly increases your learning.[1]

4. _______ **Buy** (~ $20) the **LB144 Course Pack** (buy it at SBS Bookstore). Keep it somewhere you can easily access. We will use it quite a bit, particularly when in Lab Meetings. This Course Pack contains the syllabus, lecture handouts, learning objectives, lab manual, scientific papers and lab notebook. A pdf of the Course Packet will also always be available online for easy 24/7 access.

[1] **Mueller PA, Oppenheimer DM. 2014. The Pen Is Mightier Than the Keyboard: Advantages of Longhand Over Laptop Note Taking. Psychol. Sci. 25:1159–1168.** (https://www.npr.org/2016/04/17/474525392/attention-students-put-your-laptops-away)

Week 1

(Preparing for the first lecture of the course)

Lecture1 - The LIVE in-person lecture

Budgeting homework time (50 min): Ch. 18, section 18.1 (the first half on **crickets**) is approximately 1775 words in length. At what's considered slow reading speed, 200 words per minute, reading the first half of section 18.1 should take 9 minutes. But when done properly, when you pause to review figures, read and think about a few of the Integrating Questions, and take careful notes, if you focus (avoid distraction) it should take you approx. 50 minutes.

1. _______ For the first lecture, read the 1-page **Foreword** at the beginning of the textbook written by the very famous Dr. Bruce Alberts, review the Student Resources in **Chapter 0**, and then begin reading **Chapter 18: Information in the Environment** of our book, Integrating Concepts in Biology (ICB). Read the single Introduction page. You do not need to take notes on any of these pages.

2. _______ Then slowly read the section we will discuss most during lecture, section 18.1 "Have organisms evolved to exploit communication between individuals of other species?" As you read section 18.1 (the part on crickets, not frogs) on your computer be sure to **take handwritten notes on paper in your lecture notebook**. Handwritten notes lead to much greater learning[2].

3. _______ **Try to answer at least one Integrating Question (IQ) in each set**. As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. It will help you test yourself to determine if you got the meaning, or not, while reading the last few paragraphs. Just like taking handwritten notes, this too will greatly increase your learning. If you desire a high grade in the course, try to answer more IQs.
   NOTE: Assume you will be asked a question in lecture which is directly from one of the IQs.

4. _______ **Trifecta**: **Prepare to explain (aloud) Figures 18.2, 18.3, 18.4 and Table 18.1 in class**. As you read a section from the ICB textbook always attempt to pause and study **each figure/drawing/table** that is discussed. Some of them are just pictures or drawings and may not require lots of thinking, but others are graphs or tables that contain actual data from research experiments. Spend more time looking at these. In class, during lecture, students will be randomly chosen to explain a Figure or Table aloud (LA will hand you a microphone) so prepare for when your name is called to be sure you are ready. *Some students avoid stress by just writing out in their notebook an explanation of the Purpose, Methods and Findings of each data figure* (we call these three things *the Trifecta*). If it's already written down then you can just read aloud what you wrote, like: "Purpose: Dr. Griffith wanted to determine...., Methods: his group worked with mice and pneumonia bacteria called...., Findings: in the end they found evidence that ...".

5. ________ **Advanced TIP reported from prior student**: "The way the textbook explained this figure did not make sense to me, so I scrolled down to the bottom of the page and clicked on link to the original paper and read about the same figure in that. The way the paper explained it made so much more sense and cleared up what I was confused about. Tell other students about this!"

[2] **Mueller PA, Oppenheimer DM. 2014. The Pen Is Mightier Than the Keyboard: Advantages of Longhand Over Laptop Note Taking. Psychol. Sci. 25:1159–1168.** (https://www.npr.org/2016/04/17/474525392/attention-students-put-your-laptops-away)

Week 2

Lecture2 - (Preparing for) **This week's TOP HAT lecture:**

Budgeting homework time (45 min): Ch. 18, section 18.1 (the second half on **frogs**) is 2166 words in length. At what's considered slow reading speed, 200 words per minute, reading the second half of section 18.1 should take 11 minutes. But when done properly, when you pause to review figures, read and think about a few of the Integrating Questions, and take careful notes, if you focus (avoid distraction) it should take you approx. 45 minutes.

1. _______ **For the second lecture,** slowly read the second half of section 18.1 "Frog choruses attract predators." As you read it on your computer or tablet, please be sure to **take handwritten notes on paper in your lecture notebook** (handwritten notes lead to far greater learning).
2. _______ **Try to answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. Also try to answer the green Review questions.
3. _______ (Trifecta): **Prepare to explain (aloud) Figures 18.6 and Table 18.2 in class**. As you read a section from the ICB textbook always attempt to pause and study **each figure/drawing/table** that is discussed. In LIVE Zoom classes, during lecture, you will be chosen to explain these aloud, SO on TopHat days practice, practice, practice, even stop the video and say your Trifecta aloud for the Figure or Table being discussed. Thus, you will be super ready and confident Thursdays on Zoom.
4. _______ **Advanced TIP**: scroll down to the bottom of the page, in the Bibliography, and click on the link to an original paper by Dr. Rachel Ryan to see which data was used to make figure 18.6 and Table 18.2, and look at Ulagaraj's research paper on crickets to get used to, and in a habit of, checking original papers.

Lecture3 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (60 min): In Ch. 18, section 18.3 (the second half of on **coral reefs)** is 1932 words in length which should take 10 minutes if you just read it. But when done properly, when you pause to review figures, read and think about a few of the Integrating Questions, and take careful notes, this homework assignment should take you more like 60 minutes (and that's if you are not distracted).

1. _______ **For the third lecture of the semester, read the** second half of section 18.3, after the blue header titled "Information is used by corals during settlement" and as you read it on your computer be sure to take handwritten notes*. You should focus and take detailed notes for everything about coral. Don't worry about the first part of 18.3 where the reading is about moths.
2. _______ **Try to answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. Also try to answer the green Review questions on coral reefs.
3. _______ (Trifecta): **Prepare to explain (aloud) Figures 18.18, 18.19, and 18.20 in class** (Purpose, Methods, Findings)
4. _______ **Advanced**: Click on the last reference in the Bibliography at the bottom of the page and try to find Figure 1 (not Plate 1) in Dr. Lindsay Harrington's research paper in the journal *Ecology* in 2004. Just take a peek, read the abstract and in her paper where they talk about Figure 1.

Week 3

Lecture4 - (Preparing for) **This week's TOP HAT lecture:**

Budgeting homework time (70 min): In Ch. 17, section 17.1 is 262 words, and section 17.2 (the first half on **fireflies**), is 2569 words in length, together totaling almost 2900 words. This should take 15-20 minutes if you just read it. But when done properly, when you pause to watch the three short movies, and then review a few data figures, read and think about a few of the Integrating Questions, and take careful notes, this assignment should take you 70 minutes (if you are focused).

1. _______ **For the Online lecture first read Chapter 17's,** very short, section 17.1.

2. _______Then slowly carefully read the first half of section 17.2 "How is information transmitted between members of animal species?" For section 17.2 you only need to carefully take handwritten notes in the section "Simple communication in a firefly". Be sure to watch the three short movies about the research of Dr. Sara Lewis.

3. _______ **Try to answer some Integrating Question.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

4. _______ (Trifecta): **Prepare to explain (aloud) Figures 17.3, 17.4, and 17.5 in class**. Practice, practice, even pause the video and say your TopHat Trifecta aloud. Thus, you will be very ready and very confident when called upon to speak aloud on Thursdays on Zoom.

Lecture5 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (70 min): In Ch. 17, section 17.2 (the second half of on **birds**) is 2378 words in length. Technically this should take 12 minutes if you just read it. But when done properly, when you pause to review all of the data figures and tables, read and think about a few of the Integrating & Review Questions, and take careful notes, this assignment should take you more like 70 minutes (if you are focused). **Special Allowance**: Your group can divide up the Trifectas for this lecture.

1. _______ **For the in-person lecture,** slowly read the second half of section 17.2 titled "More complex communication in a bird" (on storm petrels) and take handwritten notes.

2. _______ **Answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. Also try to answer the green Review questions.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 17.6, 17.7 and Tables 17.1, 17.2, 17.3 in class** (Purpose, Methods, Findings)

* **Special Allowance** today*: If you wish, your group can designate who will be responsible for each figure or table and thus split up the responsibility and reduce the load.

Week 4

Lecture6 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (50 min): In Ch. 17, section 17.3 (the first half on **meerkats**) is 1547 words in length. At 200 words/min this would take less than 10 minutes if you just quickly read it. But when done properly, when you pause to watch the two short movies, and then review several figures, read and think about a few of the Integrating Questions, and take careful notes, this assignment should take you 50 minutes (but longer if you are distracted by texts, friends, email etc).

1. _______ **For the Online lecture, slowly** read the first half of section 17.3 on meerkats that asks the question: "Does group living require more derived mechanisms of information transfer?". You can stop reading when you finishing reviewing Integrating Questions 25-27. Please carefully take written notes on this reading in your lecture notebook.

2. _______ **Try to answer some Integrating Question.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 17.9, 17.10, and 17.11 in class**. Practice, practice, even say your TopHat Trifecta's aloud at the appropriate time of the video. Thus, you will be very ready and very confident when called upon to speak aloud on Thursdays on Zoom.

4. _______ **Advanced**: Read on further, about Dr. Martha Manser's research on meerkats and check the Bibliography to look at one of her papers.

Lecture7 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (50 min): In Ch. 17, section 17.3 (the second half on **meerkats**) is 1180 words in length. This should take 6 minutes if you just read it. But when done properly, when you pause to watch one short movie and then review three figures, read and think about a few of the Integrating Questions, and take careful notes, this assignment should take you 50 minutes (if you are focused).

1. _______ **For the in-person lecture,** read and take handwritten notes on the second half of section 17.3 on meerkats (start after Integrating Question 27).

2. _______ (Trifecta): **Prepare to explain (aloud) Figures 17.12, 17.13, and 17.14 in class**.

3. _______ **Advanced**: Read on further, about Dr. Martha Manser's research on meerkats by looking at one of her papers in the Bibliography.

Week 5

Monday LIVE in-person:

EXAM I

Lecture8 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (70 min): Read the start of Chapter 16 and the first half of section 16.1. This is 2559 words with 6 figures; and 4 are data figures that require thinking and notetaking. Just reading the text will take 12 minutes. Yet the data figures are important. Of course, when done properly, when you pause to decipher each figure, try Integrating Questions, and take notes, this assignment will take you more like 70 minutes. **Special Allowance**: Your group can divide up the Trifectas for this lecture.

1. _______ **For the in-person lecture, first** read the first cover page of Chapter 16. Look at the Chapter location in the textbook and the Learning Objectives. No notes are necessary here.

2. _______ **Then, slowly** read the first half of section 16.1 on genes & blood pressure that asks the question: What causes individual variation? You can stop reading when you get to the blue box with the title "Variation caused by the environment". Please carefully take written notes on this reading in your lecture notebook.

3. _______ **Try to answer at least one Integrating Question in each set.** As you read the ICB textbook always attempt to test yourself a little, answer at least one IQ in each set.

4. _______ (Trifecta): **Prepare to explain (aloud) Figures 16.2, 16.3, 16.4 and 16.5 in class**.
Special Allowance today: If you wish your group can designate who will be responsible for each figure and thus split up the responsibility and reduce the load (Purpose, Methods, Findings).

5. _______ **Advanced**: Try to make sense of Table 16.1.

Week 6

Lecture9 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (50 min): Read the second half of section 16.1 titled "Variation caused by the environment". This is just 1337 words with 3 figures. Just reading the text will take 8 minutes. Yet the figures contain data, thus, when done properly, when you pause to decipher each figure, try Integrating Questions, and take notes, this assignment will take you more like 50 minutes.

1. _______ **For the Online lecture,** read the second half of section 16.1 titled "Variation caused by the environment".
2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.
3. _______ (Trifecta): **Prepare to explain (practice, do it aloud, at the appropriate time during the videos) Figures 16.6, 16.7, and 16.8** (Purpose, Methods, Findings).
4. _______ **Advanced**: Use the papers in the Bibliography to learn more, particularly if you don't quite understand something in the figures. Find out where the figure came from and go see how the author explains the results. Maybe their explanation will make more sense to you.

Lecture10 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (40 min): Read section 16.3 " Non-Mendelian genetics: Why do we need annual flu vaccines?". This is just 1889 words with 3 figures. Just reading the text will take 8 minutes. The figures do not contain data, thus, when done properly, when you pause to decipher each figure, try Integrating Questions, and take notes, this assignment will take you more like 40 minutes.

1. _______ **For the in-person lecture, slowly** read section 16.3 " Non-Mendelian genetics: Why do we need annual flu vaccines?". And please take handwritten notes.
2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.
3. _______ **Prepare to explain (aloud) Figures 16.12, 16.13, 16.14 in class**.
4. _______ **Advanced**: Check the CDC website and determine the strains of flu we currently face.

Week 7

Lecture11 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (60 min): Chapter 3, first 2/3's of section 3.1 is 2160 words in length with 7 figures that require thinking and notetaking. Reading at 200 words per minute would mean the section might take just 12 minutes to read. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 60 minutes. It could be shorter if you have been doing homework regularly, ie. training like an athlete, and getting stronger, better, faster at this now that it is week 7.

1. _______ **For the Online lecture,** read section 3.1 on Gregor Mendel titled "How can traits disappear and reappear in a later generation?" Take careful notes by hand.

2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (practice doing it aloud) Figures 3.3, 3.4, 3.6 and 3.7.**

4. _______ **Advanced**: Review how to use Punnett squares to predict the outcome of crosses.

Lecture12 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (60 min): Read and prepare for a case study on the SBE1 gene (evo-ed.org).

SBE1 case:
Read and take notes from the http://evo-ed.com/ website in the "Pea Taste" sections: Mendel to Molecules, Cell Biol, Molecular Genet, Population Genetics. *Direct link*-> http://evo-ed.com/Pages/Peas/

Use that website as well as section 3.1 on Mendel in your textbook as resources to answer these questions. Write out your answers in your paper notebook so you can photograph and turn in one copy but keep the second one for class.

Integrating Questions
1. What are Mendel's two fundamental rules of inheritance?
2. What is an allele? How do the two alleles that determine pea seed shape/taste function?
3. Why do both the RR and Rr genotypes produce round peas?
4. We call some traits dominant and others recessive, and we relate this to their respective alleles. Explain, in terms of protein function, why some traits are expressed when alleles are heterozygous.
5. Synthesis question: Does the rr genotype result in a gain or loss of function? How could either a loss or gain of function be evolutionarily important?
6. Synthesis question: Mendel and Darwin were contemporaries, although they did not know one another. How might the principles of Mendel's laws of inheritance overlap with Darwin's theory of evolution?

Week 8

Lecture13 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (60 min): Chapter 3, section 3.3 (bacterial cell division) is 2519 words in length with four figures that require thinking and notetaking for the Trifecta. Reading at 200 words per minute would mean the section might take 13 minutes to read. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 60 minutes.

1. _______ **For the Online lecture,** read Chapter 3, section 3.3 and as you read it be sure to take handwritten notes.

2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (practice doing it aloud) Figures 3.15, 3.16. and 3.17 in class** (Purpose, Methods, Findings).

4. _______ **Advanced**: Take a peek at some of the published research papers in the Bibliography at the bottom of the page with the goal to find one original figure you studied in the reading and where it is in the paper?

Lecture14 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (40 min): Chapter 3, section 3.4 (mitosis) is 2514 words in length with four photographic figures that do not require much thinking and notetaking for Trifectas. Reading at 200 words per minute would mean the section might take 13 minutes to read. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 40 minutes.

5. _______ **For the in-person lecture**, read Chapter 3, section 3.4: and as you read it be sure to take handwritten notes..

6. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

7. _______ (Tip): **Prepare to explain (aloud) what's generally happening/being explained in Figures 3.19, 3.20, 3.21 and Table 3.5 in class.**

8. _______ **Advanced**: Take a peek at some of the published research papers in the Bibliography at the bottom of the page.

Week 9

Lecture15 - (Preparing for) **This week's TOP HAT lecture:**

Budgeting homework time (40 min): Section 3.5 of **How can two parents produce non-identical offspring?** is 1931 words in length with three visual figures (yet no data figures for trifectas). Reading at 200 words per minute would mean the section might take 10 minutes to read. But when you pause to review figures and take careful notes, this assignment should take you more like 40 minutes.

1. _______ **For the Online lecture,** carefully and slowly **read** Section 3.5 of **"**How can two parents produce non-identical offspring?" and please take handwritten notes in your lecture notebook.

2. _______ **Try to answer some Integrating Questions and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Tip): **Prepare to explain (practice, doing it aloud) what's generally happening/being explained in Figures 3.22, 3.23, and 3.24.**

4. _______ **Advanced**: Take a sneak peek at the next section ELSI 3.2 on engineering better babies.

Lecture16 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (30 min): Read section ELSI 3.2 (Ethical, Legal, Social Implications): "Should we engineer better babies?" It is short, only 832 words in length, with 1 art figures (no data figures for trifectas). Reading at 200 words per minute would mean the section might take 4 minutes to read. If done properly, when you pause to take careful notes and prepare your arguments for the debate, this assignment should take you approximately 30 minutes.

1. _______ **For the in-person lecture,** read ELSI 3.2 (Ethical, Legal, Social Implications): "Should we engineer better babies?" and take handwritten notes in your notebook.

2. _______ **Try to answer the Integrating Questions**

3. _______ DEBATE Homework due at start of class: Prepare two arguments, with Claim Evidence Reasoning for each, to support your side during a debate. Use Carbonless paper (bring two copies) so you can turn one in at the start of lecture and keep the other with you.

Debate topic: Resolution -> "It is abundantly clear that we should definitely use genetic testing to eliminate genetic diseases (post-haste)!"

1. Affirmative: All students attending 12:40pm labs will be on the PRO side.
2. Negative: All students in 4:10pm labs will be on the NO side.

Week 10

Monday LIVE in-person:

EXAM II

Lecture17 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (70 min): Chapter 2, section 2.1 is 3322 words in length with three data figures that require thinking and notetaking for the Trifecta. Reading at 200 words per minute would mean the section might take 20 minutes to read. Yet figures 2.5 and 2.6 are challenging and require time to think and read about them for the Trifecta. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this will take you more like 70 minutes.

1. _______ **For the in-person lecture,** review the introductory page of **Chapter 2: Central Dogma.** Then carefully read section 2.1: "How does DNA communicate information to the cell?" and take handwritten notes in your lecture notebook.

2. _______ **Try to answer some Integrating Questions** and **Review Questions**.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 2.3, 2.5 and 2.6 in class** (Purpose, Methods, Findings).

Week 11

Lecture18 - (Preparing for) **This week's TOP HAT lecture:**

Budgeting homework time (50 min): Chapter 2, section 2.3 is that is 1725 words in length with three data figures that require thinking and notetaking for the Trifecta. Reading at 200 words per minute would mean the section might take 10 minutes to read. Yet figures 2.20 and 2.21 are challenging and require time to think and read about them for the Trifecta. Of course, when done properly, when you pause to review figures, try Integrating Questions, and take notes, this assignment will take you more like 50 minutes.

1. _______ **For the Online lecture,** carefully read section 2.3: "How do cells make proteins?" and take handwritten notes in your lecture notebook.

2. _______ **Try to answer some Integrating Questions** and **Review Questions**.

3. _______ (Trifecta): **Prepare to explain (practice, aloud) Figures 2.20, 2.21 and 2.22** (Purpose, Methods, Findings).

Lecture19 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (60 min): Chapter 2, section 2.4 is 2673 words in length with several exercises that require you to use NCBI to look for the insulin gene, find introns, and then the ORF finder to understand the gene further. Reading at 200 words per minute would mean the section might take 12 minutes to read. Yet integrating questions 35-38 are challenging and require time to think and perform what they request of you. Of course, when done properly, when you really try to do the Integrating Questions, and take notes, this assignment will take you more like 60 minutes.

1. _______ **For the in-person lecture, read another section of Chapter 2: Central Dogma.** Carefully read section 2.4: "Can cells pick and choose information?" and take handwritten notes in your lecture notebook.

2. _______ **Perform the exercises** outlined in **Integrating Questions 35-38** and then answer the remaining IQs and **Review Questions.**

3. _______ **(Tip): Prepare to demonstrate how to perform/explain (aloud) in class how to do IQs 35-38:** thus how to use online tools at NCBI and OMIM to find the DNA, RNA and amino acid sequence of any gene, with insulin as an example.

Week 12

Lecture20 - (Preparing for) **This week's TOP HAT lecture:**

Budgeting homework time (60 min): Chapter 1, has a cover page, section 1.1 is 660 words, and section 1.2 on Drs. Griffith and Avery is 1725 words in length. While this is about 2300 words in total, thus the reading would be estimated to take 12 minutes. Yet careful reading and notetaking takes time and it has four data figures. While the Trifectas are easy to prepare for, Table 1.1 may be more difficult.

1. _______ **For the Online lecture,** start **Chapter 1: Heritable Material** by reviewing the cover page and reading the short section 1.1: "What is biological information?" No notes needed.

2. _______ **Now slowly carefully read** section 1.2: "What is the heritable material?" and take careful handwritten notes in your lecture notebook.

3. _______ **Try to answer some Integrating Questions** and **Review Questions**.

4. _______ (Trifecta): **Prepare to explain (practice, aloud) Figures 1.2, 1.3, 1.4 and Table 1.1** (Purpose, Methods, Findings).

Lecture21 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (60 min): Read ELSI 1.1 and the first 2/3 of section 1.4 in "Chapter 1: Heritable Material." While this is about 3700 words in both the readings combined, only the 2700 words in section 1.4 need careful reading and notetaking. Also, there are no traditional Trifectas to prepare for, just three simple questions posed below to be ready to answer aloud in class.

1. _______ **For the in-person lecture,** revisit **Chapter 1: Heritable Material** and read section ELSI 1.1: "Who Owns Your DNA?" (975 words), and then read the first 2/3's of section 1.4 "How does DNA's shape affect its function?" Take notes on section 1.4 all the way up until it switches to the new light blue box topic of "DNA Replication" (2700 words). Read on, but don't need notes.

2. _______ **Try to answer some Integrating Questions** and **Review Questions**.

3. _______ (Tip): **Prepare to explain (aloud) in class:** 1. What's difference between the chemical structure of DNA vs RNA, 2. What's incorrect in Figure 1.10?, 3. What's incorrect in Figure 1.13?

Week 13

Lecture22 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (45 min): Chapter 1, section 1.5 on Epigenetics is 1840 words in length. At 200 words per minute, reading section 1.5 should just take 10 minutes. But when done properly, when you pause to review figures, read and think about a few of the Integrating & Review Questions, and take careful notes, this homework assignment should take you more like 45 minutes (if you focus).

1. _______ **For the Online lecture,** continue **Chapter 1: Heritable Material** by reading section 1.5: "Is all genetic information encoded linearly in the DNA sequence?" and take careful handwritten notes.

2. _______ **Try to answer some Integrating Questions** and **Review Questions**.

3. _______ **Prepare to explain (practice, aloud) Figures 1.19 (the method), and do a Trifecta for Figures 1.20, and 1.21** (Purpose, Methods, Findings).

Wednesday lecture: Journal Club, paper is TBA *to be announced*.

Thanksgiving Break

Week 14

Lecture23 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (45 min): Ch. 4, section 4.1 is about 1250 words in length and ELSI 4.1 is 1100 words thus the total is 2350 words. At 200 words per minute, reading section 4.1 & ELSI should take 12 minutes. But when done properly, when you pause to review three figures, read and think about a few of the Integrating & Review Questions, and take careful notes, this homework assignment should take you more like 45 minutes (but longer if you are distracted by texts, friends, email etc.).

1. _______ **For the lecture, read** the cover page of **Chapter 4: Evolution and Origin of Cells** in the ICB textbook, but you do not need to take notes on that page. Then slowly read section 4.1 "What is evolution?" and as you read it on your computer be sure to take handwritten notes*. Last, read the section Ethical, Legal, Social Implications (ELSI) 4.1: "Are evolution and religion compatible?" You do not need to take notes on the ELSI reading, just think about it.

2. _______ **Try to answer Integrating Question and Review Questions.** As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. Also answer the green Review questions.

3. _______ (Trifecta): **Prepare to explain (practice, aloud) Figures 4.1, 4.2 and ELSI Figure 4.1 in class**. As you read a section from the ICB textbook always attempt to pause and study **each figure/drawing/table** that is discussed.

4. _______ **Advanced TIP**: click on the link to an original version of Darwin's Origin of Species, peek at it.

Lecture24 - (Preparing for) **The LIVE in-person lecture**

Budgeting homework time (70 min): In Ch. 4, the first 2/3's of section 4.2 is 3000 words in length which should take 15 minutes if you just read it. But when done properly, when you pause to review figures, read and think about a few of the Integrating Questions, and take careful notes, this homework assignment should take you more like 70 minutes (and that's if you are not distracted).

1. _______ **Read Chapter 4's** section 4.2 "Could abiotic molecules form biologically important molecules before life evolved?" and as you read it on your computer be sure to take handwritten notes*. You should focus mostly, and only take detailed notes for, the first 2/3s of the section. You can stop taking notes once you complete the yellow Integrating Questions 5 & 6. Read the remaining section regarding RNA and directed evolution, but no notes are needed on this, just be amazed at what is said.

2. _______ **Try to answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to answer at least one of the yellow **Integrating Questions** each time you get to a set of them. Also try to answer the green Review questions.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 4.5, 4.6, and 4.8 in class** (Purpose, Methods, Findings)

4. _______ **Advanced**: Click on "Explore More on Abiotic Production of Organic Molecules".

Week 15

Lecture25 - (Preparing for) **This week's** TOP HAT **lecture:**

Budgeting homework time (60 min): In Ch. 4, the first half of section 4.3 is 2000 words in length. This should take 10 minutes if you just read it. But when done properly, when you pause to review quite a few figures, read and think about a few of the Integrating Questions, and take careful notes, this homework assignment should take you more like 60 minutes (if you are focused).

1. _______ **For the Online lecture, read Chapter 4's** section 4.3(1st half) in the ICB textbook. For section 4.3 "Can non-living objects compete and grow?" you only need to carefully read and take notes on items up to and including information related to Figure 4.13. Be sure to take handwritten notes. Then stop taking notes and just read the rest to learn about research on vesicles competing with each other. Explore Bio-Math Exploration 4.2 if you find it interesting.

2. _______ **Try to answer some Integrating Question and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (practice, aloud) Figures 4.11, 4.12, and 4.13**.

Lecture26 - (Preparing for) **The LIVE in-person lecture:**

Budgeting homework time (45 min): In Ch. 4, section 4.4 is 1500 words. This should take 8 minutes if you just read it. But when done properly, when you pause to review the two figures, read and think about the Integrating Questions, and take careful notes, this homework assignment should take you more like 45 minutes (if you are focused).

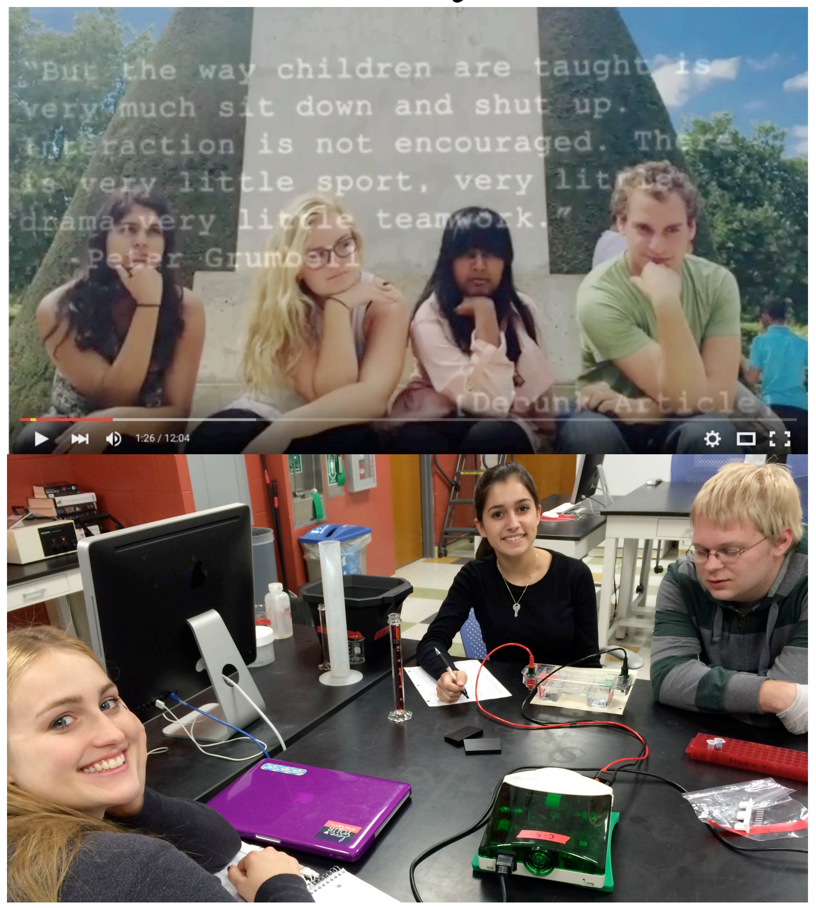
1. _______ **For the in-person lecture, read** the short reading in section 4.4 "Can non-living objects harvest and store energy?". Be sure to take handwritten notes.

2. _______ **Try to answer the two Integrating Question and Review Questions.** As you read the ICB textbook always attempt to test yourself a little, answer at least one of each set.

3. _______ (Trifecta): **Prepare to explain (aloud) Figures 4.16 and 4.17 in class**.

BIOLOGY I

Laboratory Guide



Phase I: Documenting animal communication and homologs in humans

&

Phase II: Seeking homologous genes for communication in animal and humans

Igert, Wright, Fata-Hartley, Haenisch, Cooper, Krha, Maleszewski, Wilterding, Sayed, Luckie

HYBRID EDITION

LB-144: CELL & ORGANISMAL BIOLOGY (LABORATORY)

LAB COORDINATOR

Douglas B. Luckie, Ph.D., Associate Professor, Lyman Briggs College & Dept. Physiology

LAB MANUAL

found inside "LB-144 Course Pack," (Luckie) from MSU Library Services via local bookstore

COURSE WEBSITE http://ctools.msu.edu/144

RESEARCH TEAM RATIONALE

Student groups are intended to be research & learning teams. Work with other students to study and discuss biology topics in lecture, as well as share your ideas and research predictions in lab. Teams are better learning environments but also, they are REAL LIFE. While scientists do some things on their own, they more often work in groups to solve problems because a well-functioning team is the most efficient way to work. Working in the same group in both laboratory and lecture will allow you to become more familiar with each other so you will feel comfortable enough to discuss your biology questions. Although it is easier for an instructor to run a class or lab without group work, numerous research studies have shown that working in groups and discussing science with your peers can increase your learning ***considerably*** (although you have to strive to be a "cooperative" group). By pooling your knowledge, members of your group will get "stuck" less often be able to progress far beyond what any individual in the group could do alone.

Week	Before Lab Meeting	During Laboratory Meeting Activities & Assignments DUE
1	*ONLINE LAB*	*Talking to Strangers* Film, Quiz, Honey Guide paper
2	View "IDEO" Film	Film Quiz & Debrief, Writing INTROS, Form Groups
3	View "Islands" Film	Film Quiz, 4-slide Proposal Talk & movie, Grading TITLES
4	Group Contract	***2¶-Draft due***, Preparing for LA and Prof Thesis Interviews: Q&A
5	*GEA1* on Catme.org	**LA Interviews begin** (during & outside lab time, groups of 4, 60m)
6		**LA Interviews (cont.)** Writing RESULTS & FIGURES
7		***Half-Draft due*** (2¶+ Results/Figs paper), Grading FIGURES
8	*GEA2* on Catme.org	**PCR** & **Prof Interviews begin** (during lab, in groups of 2, 60min)
9-12		Gene research (PCR, gels, Primers, BLAST =Molecular Teams)
13		***Final film*** and/or ***Final paper (DRAFT1) due***
14-15	*GEA3* on Catme.org	**Prof Interviews completed** (during lab, in groups of 2, 60min)

THE LABORATORY

You will need the Laboratory Manual resources provided in the Course Pack. Review the lab guide materials required for each week during the semester. This semester, you will design and pursue one experiment all semester long. You will find an interesting animal behavior related to communication that has been studied and published in the literature (like a mating display) and attempt to document it when observing animals on locally (like squirrels & humans). 4.0-seeking students will also connect the behavior to a gene. Your group will capture your observations with still photographs and digital video from your smartphones. Ultimately, you'll generate a short 5minute documentary film showing the results of your research and write a formal research manuscript. Each week, you will examine and practice the methods of a scientist in performing your research. This approach is aimed at mentoring you, so you master the ability to think and work like a serious scientist.

While working on group projects, you should be mindful of other students in your group; therefore, it is important for all participants to exercise:

- Respect for themselves, each other
- Openness and a positive attitude toward new ideas and other's ideas
- Flexibility and tolerance of ambiguity
- Good communications amongst themselves

ASSIGNMENT SCHEDULE & VALUES

Speaking (value)	*Writing (value)*	*Discussing/Demonstrating*
Proposal talk & movie= 10%	Proposal 2¶-Paper= 10%	LA Thesis interview= 10%
	Half-Draft Paper= 20%	Prof Thesis interview= 20%
	Final Paper/Film=30%	

Week	**Assignment(s) Due**	**Value (%)**
3	**Proposal Talk & movie**	**10**
4	**Proposal *2¶-Paper***	**10**
5	*LA Thesis Interview* (individual score, group format)	**10**
7	***Half-Draft Paper***	**20**
7-15	*Prof Thesis Interview* (individual score, pair format)	**20**
12	Final **Paper** -or- (Film option)	**30**
	Total	**= 100% of lab grade**

Tardis Pass **-**- One-Time Time Travel RE-DO permit, improve work via revision opportunity.

The "Honors Option" (optional)

*Note: The Honors Option for LB144 this semester is presenting your group's research findings as a talk at the UURAF during the Spring Semester. This is required to be an individual assignment (not done as a group) and a talk (not a poster) if you seek individual credit for an Honors Option. Be aware the UURAF application deadline is often in January.

Laboratory: Expectations & Effort

Time Commitment- "Don't be a tourist"

- Remember that the laboratory is an essential component of this class and is worth alot of points. **This means that you should be prepared to spend on average 3 hours inside the LB144 laboratory as well as 6+ hours outside of the lab with your group per week – so plan accordingly**.
- The above also means that if you explain to your research team that you are "busy" with social events every evening and intend to go home each weekend, realize you won't succeed in this course. *Do NOT be a TOURIST* - make college a priority in your life.

The LB144 laboratory is a *RESEARCH TEAM LAB*

- Your research group will need to meet twice weekly to do field research outside of class during the week as well as meet to coordinate the projects and papers you author as a group. It is essential that you read and prepare prior to coming to lab each week, because the experiments require a good amount of organization to complete in a timely manner.
- Please familiarize yourself with the concept behind this lab – it will help you to keep your "eyes on the prize" (so to speak) as the semester progresses.
- **Attendance WILL BE TAKEN** at the beginning of each lab, so it is important that you arrive ON TIME.
- The key to success in the laboratory class is to think and work like a scientist (avoid the mindset of a student simply doing the very minimum). Strive to work as though you are a professional scientist and this is your own research lab and project (which is true).

Group Roles

The research teams in LB-144 will usually consist of 4 individuals. You are expected to help out in all tasks but you will have one specialty (your primary job in the team). If your team consists of 3 individuals eliminate the "Primary Investigator" and divide those responsibilities among the team members. You will be documenting all of you biology research with audio/video. Your group creates: (1) an online research paper with figures that include graphs, photos, short clips of audio/video, and (2) a short (10m) documentary film that disseminates your research to increase public understanding of science.

-Primary Investigator *& Director* (PID) - *Plan!*

The primary investigator will be responsible for organizing meeting times, overall project planning, as well as troubleshooting throughout the investigation. It is your job to also be sure all members participate in discussions and you record notes. Ask questions when a member hasn't spoken, "What do you think, Jen?". In addition to sharing the final grade for each group paper, the PI is assigned and graded for writing specific sections of the paper as well as editorial duties on all sections. Film Project: 1° responsibility is leading the planning of the projects, storyboarding & editing of documentary film, aiding the direction of photography and audio capture.

-Protocol Expert *& Digital Editor* (PEDE) - *Protocol!*

This individual is responsible for overseeing the creation of scientific protocols for each week's independent investigation (written experiments and steps you plan to do). It is your job to worry about whether the protocol is appropriate and being followed exactly during the experiment. In addition to sharing the grade for each full draft paper, the PE is assigned and graded for writing sections of the paper as well as editorial duties on all sections in creating all complete papers. Film Project: 1° responsibility the digital editing (building) the documentary film and training others.

-Data Recorder *& Sound* (DRG) - *Record!*

The data recorded is responsible for recording and organizing the results and taking many pictures to document the team's efforts. It is your job to be sure everyone is keeping their notebook up to date and data is being recorded properly. In addition to sharing the grade for each full draft paper, the DRD is assigned and graded for writing sections of the paper as well as editorial duties on all sections in creating all complete papers. Film Project: 1º responsibility is sound recordings (audio) + digital editing of audio clips for online paper.

-Laboratory Tech *& Photography* (LTP) - *Hardware!*

This individual is responsible for learning the many experimental procedures and becoming an expert on how to use the various pieces of equipment. It is your job to read manuals and get the right equipment to work properly. In addition to sharing the grade for each full draft paper, the LT is as- signed and graded for writing certain sections of the paper as well as editorial duties on all sections in creating all complete papers. Film Project: 1º responsibility is video (photo/cinema) + editing of film clips for online paper.

Working Effectively in Small Groups

As you know, a great deal of the learning that occurs in our class is based upon your interactions with your peers in small groups. But working well in small groups does not happen by magic. There are things that I have to do as an instructor to make sure that you are getting the most out of your experience, such as identifying conflicts between group members that might hinder your work and having TAs listening closely to what you are saying as they stroll around the lab room so that we can help you get the most from your discussions or keep you on track.

You, too, have to work to make your groups all that they can be. Below, are some suggestions of effective small group work. Some of these will also clarify my expectations of you in your groups. Look this over carefully and raise questions in class or to me privately.

Individuals in Groups

There are two things that happen in small groups. Individual students share their thoughts, but those thoughts are also reacted to by one or more students. The group succeeds only insofar as each individual does actually share their thoughts; an individual who is not trying their best will diminish the group's experience. When I evaluate your group participation via GEA forms and TA feedback, I will take into account the quality of your group interactions- how well you did on your own in your group and how well the group did overall. It is up to each individual, and in particular the PI, to encourage teammates to work together to maximize your learning. If someone is not doing the reading or is not participating in lab enough, discuss the problem and see what you can do to help that person. You are all in your groups together!

Group Process

Groups are really processes, steps toward a goal. There are two types of goals toward which you are striving in our class. The first is growth as an individual. Group work should leave you feeling that you understand the material better than you could have had you worked only on your own. The second goal has to do with teamwork. Have you, and the group, learned something about group problem solving through your work together? Do you know how to better communicate with others from different backgrounds and who hold different perspectives? If so, you have achieved the second goal.

In order to make the group process work effectively, please follow these guidelines:

- Everyone should participate in your group.
- Everyone's contributions should be welcomed.
- Everyone should be heard. Listen to and hear one another; ask questions if you do not understand what a group member has said.
- No one should dominate group processes.
- A recorder should take notes for the group during a meeting. If your group meets more than once, the recorder role should be shared equally by all members through time.

Names: ____________________
____
____
____
Section #: ____________________

Team Ground Rules Contract Form

Adapted from Dr. Deborah Allen, Univ. of Delaware and Dr. Rique Campa, MSU
Team Assignment

Purpose: To establish team *norms* in order to promote constructive & productive teamwork.

Directions: To work best groups require that all team members clearly understand their responsibilities to one another. These team ground rules describe the general responsibilities of every member to the team. You can adopt additional ground rules if your group believes they are needed. Your signature on this contract form signifies your commitment to adhere to these rules and expectations.

Some questions to discuss when thinking about these rules:
1) What are your professional goals (i.e., what would you like to do following graduation)? How will working in a team help you achieve some of your professional goals?
2) Besides class time, when are you available to work with your team members (exchange your class and work schedules)?
3) What is the best method(s) and time for your team members to contact you? Share the necessary phone number(s), e-mail addresses, etc... *NOTE:* This contact information is private, so should not be shared with others outside of your team, and should only be used for class-related communication.

All group members agree to:
1. Come to class and team meetings on time.
2. Come to class and team meetings with assignments and other necessary preparations correctly and thoughtfully completed.

Additional ground rules (add as many as you like; see examples on appendix of Smith (2007)):

Example: We will always meet on these two days each week at these times ___________

If a member of the team repeatedly fails to meet these ground rules, other members of the group are expected to take the actions below. When filling in the *"If not resolved"* sections, think about how, when, and who will communicate dissatisfaction to offending team members. Reflect back on what you've learned thus far about your teammates in terms of what previous team experiences they have had, how you have worked together as a group thus far, and how each member tends to feel and deal with conflict.

Step 1: If not resolved, what will your team do? How? When?:

Step 2: Meet as a team with your lab instructor. If not resolved, what will your team do? How? When?:

Step 3: The quit or fire clause: If the steps above have been completed without resolving the problem, any team member may quit the team. Alternatively, if all other team members are in agreement, the offending team member may be fired from the team. In either case, the individual no longer working as part of a team is required to complete the remaining class activities and assignments individually.

The LB144 teaching team reserves the right to make final decisions to resolve difficulties that arise within a team. Before this becomes necessary, the team should try to find a fair and equitable solution to the problem.

Group Name:______________

Member's Names (printed), Signatures, and date:

1.____________________________

2.____________________________

3.____________________________

4.____________________________

Biology I Lab Project: Getting Started

Assignment: **Document a communication behavior in animals & then seek a homologous behavior in humans**

Build your research plan!

- [ ] 1. Find a few published research papers that document a novel behavior used by animals during communication (ideally about the same animal as you will study).

- [ ] 2. Propose a plan by which your group will document that behavior using observation and filming of your animals.

- [ ] 3. Also propose a plan to seek to detect and document that same/similar (homologous) behavior in humans.

- [ ] 4.0-level. Find a gene that may be connected to that same behavior (and might exist in both animals and humans)

- [ ] (?) Or come up with an even better idea, ie "Prof dude, we want to do something *insanely* cooler ...."

General Primers

(in this case the word *primer* means an introduction)

Primer 1: The Metric System

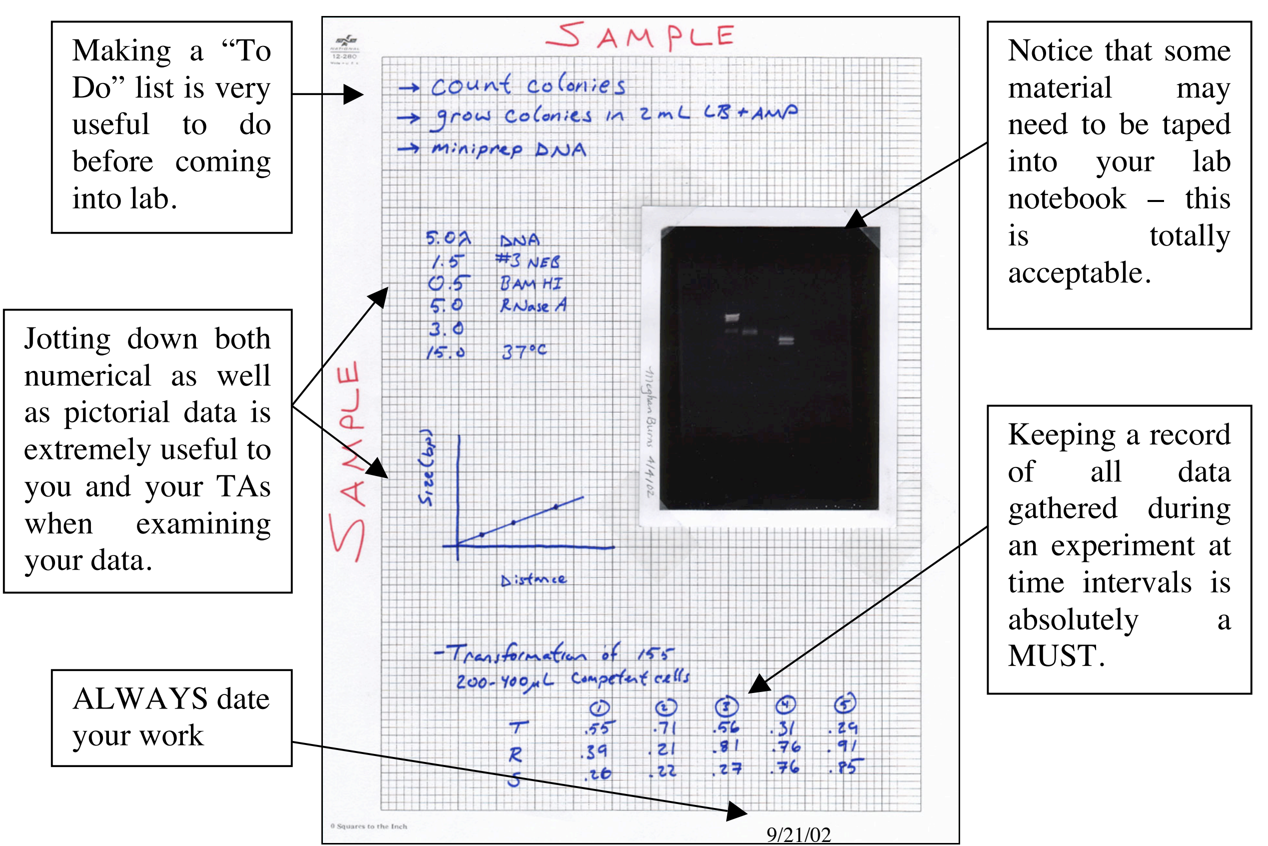
You will find it very hard to exist in lab this semester without knowing and being able to use the metric system. The short story is that a base unit (meters, grams, calories, joules, etc) is given a prefix to indicate the scale of the unit (kilo = 1000 base units; mili = 1 / 1000 base units). You should memorize at least the units in bold since they are the most likely to be used this semester and you should also be able to convert from one unit to another (ie: know how to convert 500mg into 0.5g).

List of metric prefixes, symbols, and their multipliers.			
Prefix	Symbol	Numerical multiplier	exponential
yotta	Y	1,000,000,000,000,000,000,000,000	10^{24}
zetta	Z	1,000,000,000,000,000,000,000	10^{21}
exa	E	1,000,000,000,000,000,000	10^{18}
peta	P	1,000,000,000,000,000	10^{15}
tera	T	1,000,000,000,000	10^{12}
giga	**G**	**1,000,000,000**	$\mathbf{10^{9}}$
mega	**M**	**1,000,000**	$\mathbf{10^{6}}$
kilo	**K**	**1,000**	$\mathbf{10^{3}}$
hecto	**H**	**100**	$\mathbf{10^{2}}$
deca	**da**	**10**	$\mathbf{10^{1}}$
NA		**1**	$\mathbf{10^{0}}$
deci	**d**	**0.1**	$\mathbf{10^{-1}}$
centi	**c**	**0.01**	$\mathbf{10^{-2}}$
milli	**m**	**0.001**	$\mathbf{10^{-3}}$
micro	**µ**	**0.000001**	$\mathbf{10^{-6}}$
nano	**n**	**0.000000001**	$\mathbf{10^{-9}}$
pico	p	0.000000000001	10^{-12}
femto	f	0.000000000000001	10^{-15}
atto	a	0.000000000000000001	10^{-18}
zepto	z	0.000000000000000000001	10^{-21}
yocto	y	0.000000000000000000000001	10^{-24}

Primer 2: Keeping a Laboratory Notebook

When scientists leave the lab (either to go to lunch or to go to work in another lab), their notebooks stay behind as a testament to what they did there. Other researchers in the lab may wish to know how someone had done a previous experiment, or what the results were from a different trial. Thus the notebook should be organized in such a way as to be intelligible to someone proficient in the field without any input from the author.

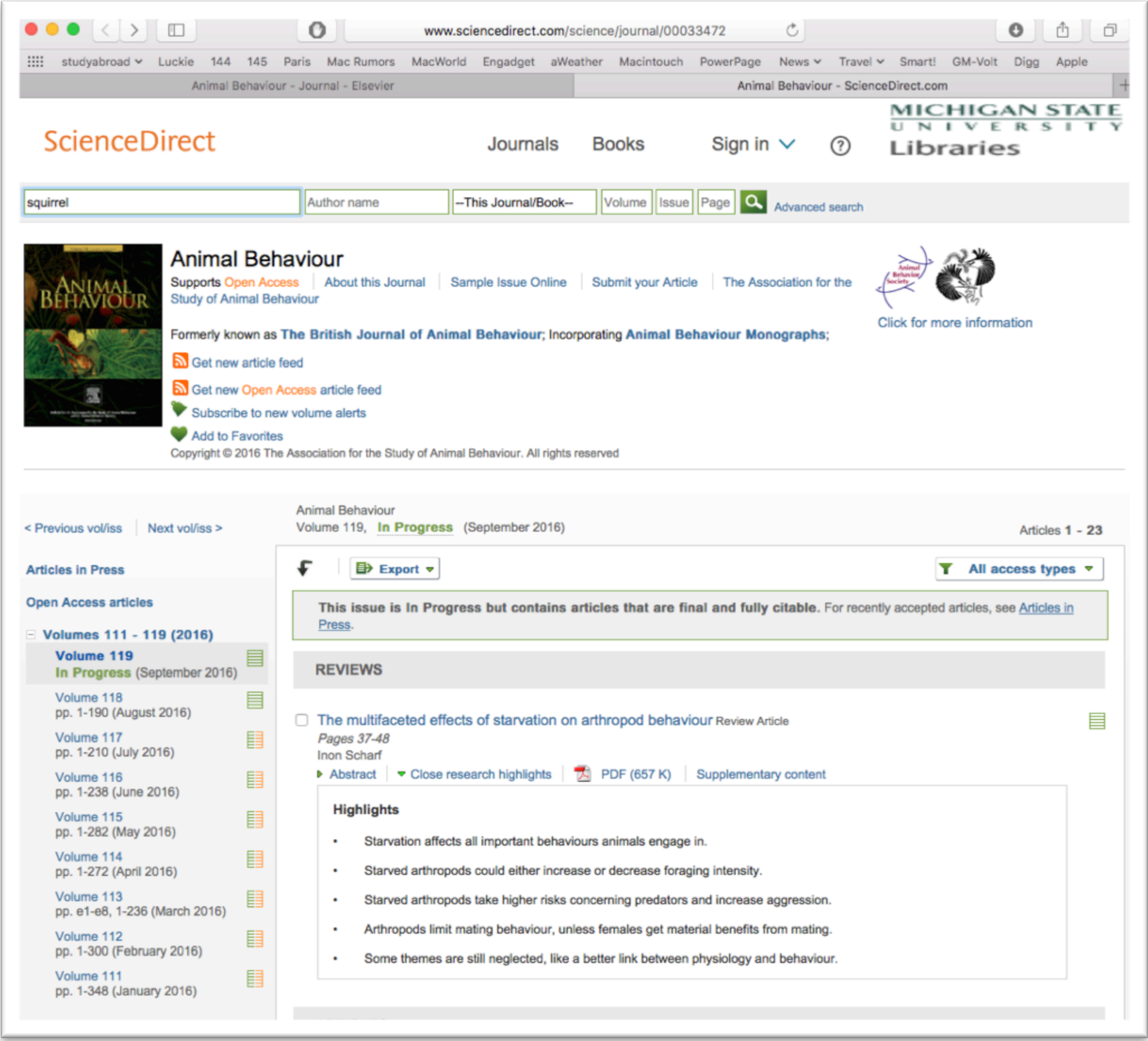
You are required to keep a notebook for LB 144. There is graph paper provided for you in the back of this notebook which will be checked to assess your notebook score (so use it as your notebook). BEFORE you leave lab each week, **You MUST get your Notebook stamped and initialed by a TA/LA.** This will be checked & graded throughout the semester and will be a means of taking attendance. Below is shown a sample page from a lab notebook. Although there is one data recorder for the group ALL students will be responsible for transferring the data to their notebooks before leaving lab. Each days entry in your notebook should include the date, the purpose of the experiment, the techniques used, and ORGANIZED data. Your time in lab will be used most effectively if you prepare as many of these elements beforehand as possible. For example, outline what your next experiment is and why you are doing it, write in the protocol or a clear reference to it (be sure to leave room for modifications), and prepare a section to enter data in (what will it look like? Do you need a graph, a table, or something else?). When a protocol is used frequently with only slight modifications, many scientists will type a copy of the protocol in a word-processor and leave blanks in which to fill in important variables (ie: make a protocol for PCR, but leave blanks for things like polymerase concentrations, primer types, etc).



Primer3

How to find published research papers on animal behavior (like squirrels)

Search Google, Google Scholar, and Animal Behavior journal websites



Primer 4: How to read a research paper like a (busy) scientist

By Candace R. Igert

At some point during the semester, you are going to need to read a research paper. If you are striving to do well in this course, you will likely be reading a lot of research papers or journals. Just like everything else in science, there is an effective method to make this less painful and time-consuming. Below is a set of steps an experienced scientist will take when reading a journal or even deciding if it is relevant to what they are looking for. The scientist's goal is getting important information fast. They do not read from beginning to end, but skip around.

Step 1: **A scientist will read the title.** This may sound simple and kind of silly to have it as a step, but the title of a paper can tell you what species/cells were used, what technique was used, what was found, and/or what the research aims were. Sometimes, by this information alone, you can decide if a paper is useful or relevant.

Step 2: **A scientist will then look at the authors and whom they are affiliated with.** This step can help to give credibility to the article or to give you a better idea of what the paper is about. If after reading X amount of journals on CF, you may know that Dr. Smith is an expert on only one aspect of CF or perhaps Dr. Smith works at Harvard, his lends credibility to his/her work. Scientists read slowly and carefully when they determine if the paper is important and/or done by outstanding people.

Step 3: **A scientist will now read critically read the abstract.** At this point, a scientist would read the abstract and glean as much information from it as possible such as the research question, the hypothesis, the predictions, the methods used, the outcome, and how it is interpreted (in other words, what does all of it mean?). All of these aspects are not always apparent or present in every abstract as each journal has different requirements in layout. A great abstract will often share the most important findings and data.

Step 4: **A scientist will read the Introduction if not familiar with the topic.** If the subject that this journal focuses on is not one that you are familiar with, reading the introduction is a way to quickly get yourself up to speed. Perhaps you are familiar with the topic then read the introduction until you catch yourself shaking your head in confirmation or going "Yea, I know this."

Step 5: **A scientist will then go on to look at the figures and tables.** Using the figure and table legends, which are usually pretty extensive and descriptive, a scientist will try to discern what is shown in each figure including what it means in terms of the research aims and interpret data presented in tables. If you come upon something you do not understand by just reading the legend, proceed to Step 5.

Step 6: **A scientist will then read the text to clarify.** When a scientist finds that s/he does not understand a figure or table and needs further explanation, s/he will locate where that figure is referenced in the text and read that portion for

clarification. S/he will first just scan the Results section to find where that figure or table is cited e.g. "(Figure 3)".

Step 7: **<u>When relevant, a scientist will finally read the discussion.</u>** After reading the title, looking at the authors and their affiliates, critically reading the abstract, and understanding the figures, you may wonder what they concluded from all of that, what they think went wrong/can be fixed in the future, or where they plan to take their research in the future. If this is the case then read the discussion/conclusion.

What information you gain or want from a paper will vary depending on your research needs at that point in time. If you are looking for a primer sequence, then you may skip Step 4 and read the Methods sections to see if their sequence is listed. As you learn how to read papers, you will also learn how to effectively customize the process depending on the occasion.

** Disclaimer: If asked to read a research paper for a class, it is probably best not to only employ this method.

Primer5 BE JANE

Try to learn from Jane Goodall's work *(read more about her online)*

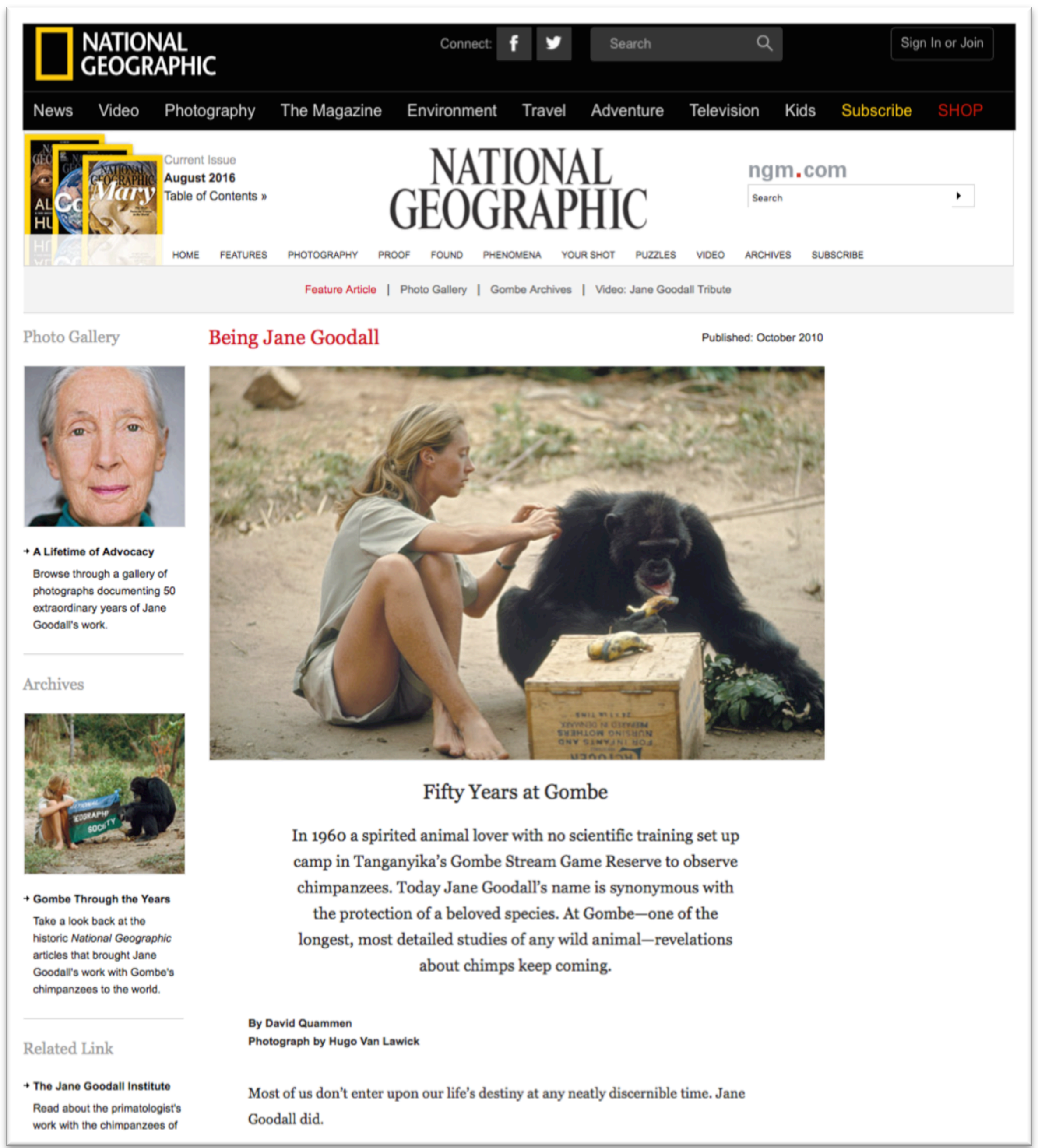


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Table of Contents »

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Feature Article | Photo Gallery | Gombe Archives | Video: Jane Goodall Tribute

Photo Gallery

+ A Lifetime of Advocacy

Browse through a gallery of photographs documenting 50 extraordinary years of Jane Goodall's work.

Archives

+ Gombe Through the Years

Take a look back at the historic *National Geographic* articles that brought Jane Goodall's work with Gombe's chimpanzees to the world.

Related Link

+ The Jane Goodall Institute

Read about the primatologist's work with the chimpanzees of

Being Jane Goodall

Published: October 2010

Fifty Years at Gombe

In 1960 a spirited animal lover with no scientific training set up camp in Tanganyika's Gombe Stream Game Reserve to observe chimpanzees. Today Jane Goodall's name is synonymous with the protection of a beloved species. At Gombe—one of the longest, most detailed studies of any wild animal—revelations about chimps keep coming.

By David Quammen
Photograph by Hugo Van Lawick

Most of us don't enter upon our life's destiny at any neatly discernible time. Jane Goodall did.

Primer 6

Making video: Nature and wildlife | Life and style | The Guardian 8/7/16

Nature and wildlife

Be it Terry Nutkins, David Bellamy or the late Steve Irwin, everyone has a favourite nature documentary presenter, and most love watching critters from the animal kingdom going about their daily business. (For proof, check out how many people have viewed the legendary "Battle at Kruger" on YouTube.)

Nowadays, there's no reason you can't have a go at creating your own nature documentary. Even with amateur equipment, it's possible to capture extraordinary footage and transform it into an insightful short film without having to book a safari or risk being spat at by a King Cobra. What matters most is to have background knowledge of what you are filming, to know what type of camera moves will suit your subject, and to make the narrative exciting and energetic. Master these basics and you could be well on your way to becoming the next, er, Michaela Strachan.

What to shoot

"Wildlife and nature filming is unpredictable, but the challenge is part of the fun," explains presenter Eleni Andreadis from green.tv, a broadband TV channel for environmental films.

At green.tv, two nature videos are uploaded each week. The footage is no longer than five minutes, it's engaging and packed with information. To create something similar at home, first do some planning and thoroughly research the animal's behaviour. Remember that the subject itself (cute though it may be) won't be enough to create an engaging short film. To make something watchable, you'll also need an informed presenter (which could be yourself) and/or an expert you can interview on screen.

How to prepare

Nature videos are not heavily "storyboarded", but they do need a structure to keep viewers interested. Because of the unpredictablity of the subject , you're going to have to improvise as you shoot, but it's still wise to start with a plan and then adapt it when necessary.

First, think about the aim of your nature video: is it a documentary or a short film? Do you need to interview people? Also, think about the conditions you are likely to be filming in. You'll probably be outside in natural light, so the limitations of your equipment will need to be taken into account.

Planned walks in parks and wetland centres are a good starting point, as the animals are more contained and easier to film. If you are going on a tour, check it's OK for you to film, and factor in the tour's pace. If it's led by an expert, ask if you can interview them. Above all, research everything (and double-check it) before you start filming -

particularly if you are making a more political film about the effects of climate change, say. Without an informed commentary to support your images, the film will be lightweight.

On the shoot

"Let the animal walk out of the shot," advises green.tv's Verity Cowper. "Film the spot you think they are headed towards, and get them coming into and then leaving the frame. This gives your footage a start and end when you come to edit a sequence together - just make sure there's a series of shots at different focal lengths [not necessarily all taken in sequence] edited between these two to avoid the footage being dull for viewers."

The pull focus shot works well in nature documentaries as it helps to put your subject in context and draw in your audience.

If you are using a presenter in the footage, make sure there's a balance between the amount of nature and presenter shown. And if the footage is going online, it really needs to be short and succinct.

Equipment

If you are filming in unreliable weather, you'll probably want to invest in a rain cover for your camera. These are widely available from £70 (creativevideo.co.uk). An external mic for your presenter or interviewee will add finesse.

What not to do

You'll be shooting unpredictable subjects outdoors, so get familiar with your kit. Don't invest in artificial lighting or try techniques you're not confident with. By the time you've worked out the shot, your animal will have vanished out of sight! Also, allow your camera to acclimatise if you're coming in from the cold: you won't see a thing through a steamed- up lens.

Tips and techniques

A quirky tip is to watch Lion King! "Even though it's animated, that film offers a great example of the different focal lengths useful in nature videos," says Verity.

Resources

· green.tv/wwt View the wide range of wildlife and other eco-shorts - and even apply to be a presenter!

· wildlife-film.comInternational site offering training courses, stock footage and more

Primer 7

Getting started with HTML

http://www.w3.org/MarkUp/Guide/Overview.html

by Dave Raggett

This is a short introduction to writing HTML. What is HTML? It is a special kind of text document that is used by Web browsers to present text and graphics. The text includes markup tags such as <p> to indicate the start of a paragraph, and </p> to indicate the end of a paragraph. HTML documents are often referred to as "Web pages". The browser retrieves Web pages from Web servers that thanks to the Internet, can be pretty much anywhere in World.

Many people still write HTML by hand using tools such as NotePad on Windows, or TextEdit on the Mac. This guide will get you up and running. Even if you don't intend to edit HTML directly and instead plan to use an HTML editor such as Netscape Composer, or W3C's Amaya, this guide will enable you to understand enough to make better use of such tools and how to make your HTML documents accessible on a wide range of browsers.

***p.s.** a good way to learn is to look at how other people have coded their html pages. To do this, click on the "View" menu and then on "Source". On some browsers, you instead need to click on the "File" menu and then on "View Source". Try it with this page to see how I have applied the ideas I explain below. You will find yourself developing a critical eye as many pages look rather a mess under the hood!

For Mac users, before you can save a file with the ".html" extension, you will need to ensure that your document is formatted as plain text. For TextEdit, you can set this with the "Format" menu's "Make Plain Text" option.

This page will teach you how to:

- start with a title
- add headings and paragraphs
- add emphasis to your text
- add images
- add links to other pages
- use various kinds of lists

Start with a title

Every HTML document needs a title. Here is what you need to type:

```
<title>My first HTML document</title>
```

Change the text from "My first HTML document" to suit your own needs. The title text is preceded by the start tag <title> and ends with the matching end tag </title>. The title should be placed at the beginning of your document.

To try this out, type the above into a text editor and save the file as "test.html", then view the file in a web browser. If the file extension is ".html" or ".htm" then the browser will recognize it as HTML. Most browsers show the title in the window caption bar. With just a title, the browser will

show a blank page. Don't worry. The next section will show how to add displayable content.

Add headings and paragraphs

If you have used Microsoft Word, you will be familiar with the built in styles for headings of differing importance. In HTML there are six levels of headings. H1 is the most important, H2 is slightly less important, and so on down to H6, the least important.

Here is how to add an important heading:

```
<h1>An important heading</h1>
```

and here is a slightly less important heading:

```
<h2>A slightly less important heading</h2>
```

Each paragraph you write should start with a <p> tag. The </p> is optional, unlike the end tags for elements like headings. For example:

```
<p>This is the first paragraph.</p>
<p>This is the second paragraph.</p>
```

Adding a bit of emphasis

You can emphasize one or more words with the <em> tag, for instance:

```
This is a really <em>interesting</em> topic!
```

Adding interest to your pages with images

Images can be used to make your Web pages distinctive and greatly help to get your message across. The simple way to add an image is using the <img> tag. Let's assume you have an image file called "peter.jpg" in the same

folder/directory as your HTML file. It is 200 pixels wide by 150 pixels high.

```
<img src="peter.jpg" width="200" height="150">
```

The src attribute names the image file. The width and height aren't strictly necessary but help to speed the display of your Web page. Something is still missing! People who can't see the image need a description they can read in its absence. You can add a short description as follows:

```
<img src="peter.jpg" width="200" height="150"
alt="My friend Peter">
```

The alt attribute is used to give the short description, in this case "My friend Peter". For complex images, you may need to also give a longer description. Assuming this has been written in the file "peter.html", you can add one as follows using the longdesc attribute:

```
<img src="peter.jpg" width="200" height="150"
alt="My friend Peter" longdesc="peter.html">
```

You can create images in a number of ways, for instance with a digital camera, by scanning an

image in, or creating one with a painting or drawing program. Most browsers understand GIF and JPEG image formats, newer browsers also understand the PNG image format. To avoid long delays while the image is downloaded over the network, you should avoid using large image files.

Generally speaking, JPEG is best for photographs and other smoothly varying images, while GIF and PNG are good for graphics art involving flat areas of color, lines and text. All three formats support options for progressive rendering where a crude version of the image is sent first and progressively refined.

Adding links to other pages

What makes the Web so effective is the ability to define links from one page to another, and to follow links at the click of a button. A single click can take you right across the world!

Links are defined with the <a> tag. Lets define a link to the page defined in the file "peter.html" in the same folder/directory as the HTML file you are editing:

This a link to Peter's page.

The text between the <a> and the is used as the caption for the link. It is common for the caption to be in blue underlined text.

If the file you are linking to is in a parent folder/directory, you need to put "../" in front of it, for instance:

Mary's page

If the file you are linking to is in a subdirectory, you need to put the name of the

subdirectory followed by a "/" in front of it, for instance:

Sue's page

The use of relative paths allows you to link to a file by walking up and down the tree of directories as needed, for instance:

John's page

Which first looks in the parent directory for another directory called "college", and then at a subdirectory of that named "friends" for a file called "john.html".

To link to a page on another Web site you need to give the full Web address (commonly called a URL), for instance to link to www.w3.org you need to write:

This is a link to W3C.

You can turn an image into a hypertext link, for example, the following allows you to click on the company logo to get to the home page:

<img src="logo.gif" alt="home page">

This uses "/" to refer to the root of the directory tree, i.e. the home page.

Three kinds of lists

HTML supports three kinds of lists. The first kind is a bulletted list, often called an ***unordered list***. It uses the <ul> and <li> tags, for instance:

```
<ul>
  <li>the first list item</li>
  <li>the second list item</li>
  <li>the third list item</li>
</ul>
```

Note that you always need to end the list with the end tag, but that the is optional and can be left off. The second kind of list is a numbered list, often called an ***ordered list***. It uses the <ol> and <li> tags. For instance:

```
<ol>
  <li>the first list item</li>
  <li>the second list item</li>
  <li>the third list item</li>
</ol>
```

Like bulletted lists, you always need to end the list with the end tag, but the end tag is optional and can be left off.

The third and final kind of list is the definition list. This allows you to list terms and their definitions. This kind of list starts with a <dl> tag and ends with </dl> Each term starts with a <dt> tag and each definition starts with a <dd>. For instance:

```
<dl>
  <dt>the first term</dt>
  <dd>its definition</dd>
  <dt>the second term</dt>
  <dd>its definition</dd>
  <dt>the third term</dt>
  <dd>its definition</dd>
</dl>
```

The end tags </dt> and </dd> are optional and can be left off. Note that lists can be nested, one within another. For instance:

```
<ol>
  <li>the first list item</li>
  <li>
    the second list item
    <ul>
      <li>first nested item</li>
      <li>second nested item</li>
    </ul>
</li>
```

```
  <li>the third list item</li>
 </ol>
```

You can also make use of paragraphs and headings etc. for longer list items.

HTML has a head and a body

If you use your web browser's view source feature (see the View or File menus) you can see the structure of HTML pages. The document generally starts with a declaration of which version of HTML has been used, and is then followed by an <html> tag followed by <head> and at the very end by </html>. The <html> ... </html> acts like a container for the document. The <head> ... </head> contains the title, and information on style sheets and scripts, while the <body> ... </body> contains the markup with the visible content. Here is a template you can copy and paste into your text editor for creating your own pages:

```
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
    "http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
  <title> replace with your document's title </title>
</head>
<body>

replace with your document's content

</body>
</html>
```

Tidying up your markup

A convenient way to automatically fix markup errors is to use HTML Tidy which also tidies the markup making it easier to read and easier to edit. I recommend you regularly run Tidy over any markup you are editing. Tidy is very effective at cleaning up markup created by authoring tools with sloppy habits. Tidy is available for a wide range of operating systems from the TidyLib Sourceforge site, and has also been integrated into a variety of HTML editing tools.

Getting Further Information

If you are ready to learn more, I have prepared some accompanying material on advanced HTML and adding a touch of style.

W3C's Recommendation for HTML 4.0 is the authoritative specification for HTML. However, it is a technical specification. For a less technical source of information you may want to purchase one of the many books on HTML, for example "Raggett on HTML 4", published 1998 by Addison Wesley. XHTML 1.0 is now a W3C Recommendation.

Best of luck and get writing!

Dave Raggett <*dsr@w3.org*>

Primer 8: Examples of how to write an email to a researcher

Here are some examples of the style and wording you should go for when emailing a scientist to seek a sample of genomic DNA with your particular mutation.

Your goal is to establish an authentic one-on-one conversation between two peer researchers. Be a scientist not a student. Be sure to send the email from your msu.edu email account. They will look at that first. It establishes credibility.

Dear Dr. X,

I just read your article in The Canadian Journal of Neurological Sciences on dystrophin mutations and seek your advice. I'm currently designing a customized PCR assay to detect the deletion of exon 47 on the dystrophin gene and I'm curious if there is anyone in the field you might recommend I contact to obtain a small sample of genomic DNA with the mutation to serve as a control for testing my assay.

Any help you can send my way would be greatly appreciated.

Sincerely,

Kim Vi
MSU Diagnostics Lab
Michigan State University
vikim@msu.edu

Dear Dr. Fang,

I'm studying the SMN1 gene and just read your 2012 article in Biomedcentral Medical Genetics on SMN1 gene mutations in SMA patients of Chinese descent. I'm currently designing a diagnostic assay for the Arg288Met mutation in SMN1 using PCR. I'm contacting you for advice. Do you know of anyone in the field who I might contact to obtain a small sample of genomic sequence to serve as a positive control in my work?

Any help you could send my way would be greatly appreciated.

Best Regards,

Rajvinder Singh
Research Laboratory of Natural Science
Michigan State University

THE ELEMENTS OF A GOOD HYPOTHESIS

The American Heritage Dictionary defines hypothesis as "*a tentative explanation for an observation, phenomenon, or scientific problem that can be tested by further investigation*". A hypothesis is a possible answer to a question, from which predictions can be made and tested. There can be multiple hypotheses used to answer a single question and for each hypothesis, multiple predictions can usually be made.

The foundation for high quality, biological research is a good hypothesis. A good hypothesis is more than just an educated guess.

THE HYPOTHESIS SCORE CARD…

A good hypothesis must:

1.) explain how or why: provide a mechanism

2.) be compatible with and based upon the existing body of evidence.

3.) link an effect to a variable.

4.) state the expected effect.

5.) be testable.

6.) have at least two outcomes.

7.) have the potential to be refuted.

Hypotheses can be scored based on these elements. When considering a hypothesis, give one point for each of the elements. An *accomplished hypothesis* will have a score of 7. An *incomplete or developing hypothesis* will have a score of 5-6. A score below 5 is an *attempted hypothesis* or *not a hypothesis*. You should use this scoring procedure when developing your own hypotheses or when evaluating hypotheses of others.

Writing Information

Instructions to Authors

Follow these instructions or your paper will be returned to you, and incur late penalties.

One of the learning objectives of your research project in the course is to develop your scientific writing skills. In science, writing is the most important means of communicating research findings. Major scientific findings are rarely kept secret. Instead, scientists share their ideas and results with other scientists, encouraging critical review and alternate interpretations from colleagues and the entire scientific community. In most cases, scientists report the results of their research activities in scientific journals in a standard written format. In this course, you will practice writing using this same standard scientific format and style.

4.0 TIP: Write like a scientist. Write your papers so that anyone who reads your manuscript could not tell it was from a student, but assumes it must have been from a scientist in a lab at MSU.

A scientific paper includes the following: a **TITLE** (statement of the question or problem), an **ABSTRACT** (short summary), an **INTRODUCTION** (background and significance of the problem), a **METHODS** section (report of exactly what you did), a **RESULTS** section (presentation of data), a **DISCUSSION** section (interpretation and discussion of your results), and **REFERENCES** (books and periodicals used). Data is also represented by **FIGURES** and **TABLES**.

Throughout the laboratory, you will practice scientific research and writing. Your papers will be reviewed by the course professor, TAs, and your peers in order to point out your areas of weakness and make suggestions for future improvements. By the time you have completed the course, you will have submitted the equivalent of two full scientific papers. If you are not certain about the level of independence and what constitutes plagiarism in this program, ask your instructor to clarify the class policy. ***Plagiarism will not be taken lightly and will be evaluated by instructors and software at turnitin.com. See syllabus for more info.***

Predictions: Science is not about explanation, but in fact it is about the ability to predict. All scientists must have models or hypotheses that can be used to then make predictions of what will occur. Thus prediction is a very important part of writing you papers. As a result in your early DRAFT1 and DRAFT2 in many cases your predictions may be all the data you have on a certain experiment and thus they should be well supported by papers from the literature. Use future tense when discussing Predictions. In general whether it's in the Abstract, Introduction, Results or Discussion, whenever you discuss something that you predict it should be in the format of:

"We predict..[what].. because..[rationale].. (citation of paper)."

**Note: A hypothesis is different than a prediction. The hypothesis is the model that explains how you believe things are working (e.g. we hypothesize electricity sparks gas in the cylinders of a car engine) while the prediction is what you think should happen during the experiment (e.g. if hydrogen gas is injected into the cylinder of a functioning engine we predict there should be a significant explosion).

Overview of Manuscript Sequence & Format
(This is what professional journals expect)

1. Page one is the Title Page (≤100 characters in title)
2. Page two will have ONLY the Abstract (≤250 words)
3. Page three will start with the Introduction. The Methods, Results, and Discussion sections can follow without starting a new page for each one (although you may start a new page if you are near the bottom of the current page). Clearly label each section with the section headings (ex. Introduction) and who authored it (Written by: Jill Sanders, Revised by: Bob Roberts).
4. The **Introduction** will provide the reader with the background information necessary to understand the rest of the paper.
5. **Methods** section will list materials used (Bought Vitamin C with Rose hips from the General Nutrition Store (GNC) 324 E. Grand River East Lansing MI) how stock solutions were prepared and explain exactly what you did in your research. After reading the Methods section, an incoming student should be able to repeat your work. Reference the original protocol.
6. The **Results** section follows Methods. This section will clearly and succinctly state what you observed upon performing each experiment.
7. The **Discussion** section follows Results. In this section you will discuss the significance of results and how your results relate with research performed by others.
8. The **References** section follows the Discussion. This is a list of the references cited within the paper.
9. Start a new page with the **Figures** section after the References. Figures will be sequentially numbered in the order that they were cited in the Results section (figures are most always cited ONLY in the Results section, not in Methods, not in Discussion). One figure per page with extensive figure legend paragraph ONLY at the bottom of the figure. The first sentence of a figure legend is its title. Follow the title with sentences explaining the figure as if someone did not have the Results section or in fact any other part of the paper available as a reference.
10. The **Tables** section will follow the Figures section. Tables get a title ONLY on the top with some explanation. Tables will be sequentially numbered in the order that they were cited in the Results section (Tables are most always cited ONLY in the Results). One table per page.
11. Figures and tables MUST be created on a computer unless otherwise instructed.
12. After the Tables section, a single white page will follow entitled, **Appendix**. Then append any laboratory notebook pages that indicate signed data for all members (photocopies from your notebook) and a photocopy of the first page of any articles cited and referenced in report.
13. Double space or 1.5 space typeface is required. Preferred font size is 12 point.

***Once returned, rejected papers (like late papers) lose one point in the first 24 hrs grace period but then the penalty becomes more severe: 10% off for 2 days late, 20% off for 3 days, and so on. After 5 days, you will receieve a "0". Unlike late papers, a rejected paper also loses 1 pt per rejection.**

A more detailed description of each section of a scientific paper follows also, review the published papers provided in the course packet for examples. As you write your paper, clearly label each section (except the title page), placing the title of the section on a separate line, centered, bold, but not underlined (like shown below).

Title Page and Title

The title page is the first page of the paper and includes the title of the paper, your name, the course title, your lab time, your lab instructors' names, the due date for the paper, and your groups' website address. The title should be as short as possible and as long as necessary to communicate to the reader the question being answered in the paper. Consider the following titles for a paper that describes the molecular mechanism of an antiviral drug.

1. "Inhibition of Mengovirus Replication by Dipyridamole"
2. "Antiviral Action of Dipyridamole"
3. "A Study Examining the Inhibitory Effects of the Drug Dipyridamole on Mengovirus Replication"

Title 1 is short and communicates the question being investigated. It conveys the mechanism of action (inhibition of replication), the name of the virus being inhibited (Mengovirus), and the name of the drug doing the inhibiting (Dipyridamole). Title 2 is short but too vague for the reader to know the subject matter of the paper. Title 3 is too long. The words "A Study Examining" are superfluous, and "Drug" and is redundant.

Place the title about 7 cm from the top of the title page. Place "by" and your name(s) in the center of the page, and place the course title, lab time, lab instructors' names, due date, and your groups' website address, each on a separate centered line, at the bottom of the page. Leave about 5 cm of white space below this information.

Abstract

The abstract is placed at the beginning of the second page of the paper, after the title page. The abstract summarizes the question being investigated in the paper, the methods used in the experiment, the results, and the conclusions drawn. The reader should be able to determine the major topics in the paper without reading the entire paper. As mentioned previously, predictions are an essential element of science and thus should appear in the Abstract of DRAFT1 and DRAFT2 and in the format: ***"We predict..[what].. because..[rationale].. (citation of paper)."***

Introduction

Start the introduction on page three. The introduction should generally be short, only 4-5 paragraphs in length and focus are background information of the following types:

1. Describe the question and hypothesis being investigated and background on the importance of the topic.
2. Review the background information that will allow the reader to understand the purpose and topics of the paper. There is usually a paragraph on the specimens studied, also one that provides evidence to support the hypothesis posed. A hypothesis is an educated guess; the Introduction should provide the "education." Include only information that directly prepares the reader to understand the question investigated. Most of this information should come

from outside sources, such as scientific journals or books dealing with the topic you are investigating.*

3. In a paragraph state background information on the methods chosen to investigate the hypothesis. Explain how these methods will address the question and describe the predicted outcomes. Why were they chosen?
4. In the last paragraph briefly state a hint of the results and conclusions of the investigations (or predictions). This generally comes only at the very end of the Introduction.

*All sources of information must be referenced and included in the References section of the paper, but the introduction must be in your own words. ***No "quotations" are permitted in any part of the paper.*** Refer to the references when appropriate. As you describe your investigation, include only the question and hypothesis that you actually investigated. It is a good idea to write down each item (question, hypothesis, supporting evidence, prediction) before you begin to write your introduction.

Write the introduction in past tense when referring to elements of your experimental investigation that are completed. When relating the background information, use present tense when referring to another investigator's published work. Use future tense when discussing Predictions.

Methods

The Methods section describes your experiment in such a way that it may be repeated exactly. Make the Methods professional just like in published papers, but target as your audience a student in LB-144. The majority of the information in this section comes from the Procedures or Protocols section of the Laboratory Guide and in your paper, this information should not be a list of steps. Write the Methods section in a paragraph format in past tense. Be sure to include levels of treatment, numbers of replications, and control of treatments. If you are working with living organisms, include the species and the sex of the research organism. Do not include failed attempts unless other investigators may wish to try the technique used. Do not try to justify your procedures in this section of the report.

If you describe an experiment from the lab guide, unless instructed otherwise, you may simply refer to the procedures listed in the guide (and page numbers). Under those circumstances, your Methods section should point out changes in procedure that are not indicated in the Lab Manual. When writing a full Methods section (with no reference to the Lab Guide), write these procedures concisely, but in paragraph form. The difficulty comes as you decide the level of detail to include in your paragraphs. You must determine which details are essential for the investigator to repeat the experiment. For example, if in your experiment you incubated potato pieces in different concentrations of sucrose solution, it would not be necessary to explain that the pieces were incubated in plastic cups labeled with a wax marking pencil. In this case, the molarity of the sucrose solutions, the size of the potato pieces and how they were obtained, and the amount of incubation solution are important items to include

Results

The Results section consists of two components: (1) one or more paragraphs that describe the results of each experiment/test and include the actual data with observations, specific numbers [and units] and math, (2) reference to figures (graphs, diagrams, pictures), and reference to tables.

In referencing figures, remember to number figures and tables consecutively in the order that they are mentioned in this section. Refer to figures and tables within the paragraph as you describe your results, using the word Figure or Table in parentheses, followed by its number, for example, "(Figure 1)." Avoid citing a figure with a full sentence or statement such as, "please see figure 1 for graphed data points" or even just "please see table 2." DO NOT place each figure or table at the end of each paragraph in which it is cited. Place figures and tables after the References section. If you have performed a statistical analysis of your data, such as chi-squared, include this data in the Results section. Explain what calculations you did and the result and direct the reader to the Methods section for more details. Then show an example of the results and refer the reader to a table with all the data.

The most common error a student makes is only making general comments and not including actual data (ie the results) in the Results. Results should include detailed observations (what did you see: odor, texture, etc.) and specific findings (what did you record: 15 grams, 20 degrees C, 20% increase, 32,000 cells etc.). Report your data as accurately as possible in the order that they happened. It is important to have separate paragraphs and topic sentences that introduce the results of each test but do not spend much time discussing the meaning of your findings, save that for the Discussion.

For "***Predicted Results***" - which will be the only focus of Draft 1 and will be included in subsequent drafts - these are what you predict or expect will happen, and these predictions must be based upon the primary literature you have gathered throughout your research on your topic/thesis. When including predictions follow this format: ***"We predict..[what].. because..[rationale].. (citation of a published paper or source)."***

Discussion

The Discussion section is where you will analyze and interpret the results of your experiments. The Discussion should show a possible relationship between observed facts—those observed by you and those observed by others and reported in published research papers. Write as clearly and succinctly as possible. A good Discussion will include the following:

1. Summarize the Introduction and restate the question and hypothesis being addressed.
2. Briefly summarize the results of the experiments. Do not include details regarding methods.
3. Interpret the results. Explain how the results answer the questions posed. State whether your results support or refute your hypothesis. Do not use the word "prove" in your conclusions. Your results will support, verify, or confirm your hypothesis. They also may negate, refute, or contradict your hypothesis. The word prove is not appropriate in scientific writing.
4. Discuss how your results and interpretations relate with previously published research. This will require you to cite outside references. Some may come from the Introduction, while you will also find new references that specifically relate to your findings. You can speculate and propose theoretical implications of your work.

5. Describe weaknesses in experimental design or technical difficulties that arose during the research. Explain how these problems specifically affected the outcome of the research. Any human errors (spills, etc) discussed should have been then corrected by certain steps.
6. Discuss experiments that would be performed if the research were to be continued. Explain how those experiments would contribute to answering the questions addressed by the research.

Figures

All figures should be computer generated. The format of the figure will depend on the type of data collected. Your figures will include mostly photographs and graphs. The photos and graphs must be done in a professional manner and include computer generated labels when appropriate, and always with only one figure per page. Under each photo or graph, there must be a legend paragraph. The legend paragraph will include the Figure number, a title sentence, and a description of what was done in the experiment and shown in different labeled parts of the figure. A reader must be able to understand the general concept of the experiment performed without reading the Methods section. In fact if a student from LB-144 picks up a single piece of paper with one of your figures on it, they should be able to explain to you what that experiment is about from the legend.

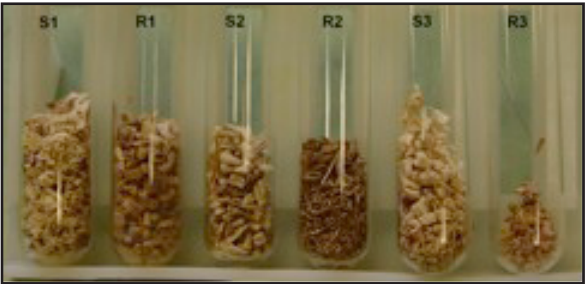

Figure 1. Preparation of corn root and stalk samples for sugar analysis. Samples were cut into units no greater than 4 mm3 using a surgical scalpel. Three stalk samples of 70 grams each and three root samples of 80 grams each were prepared. Root 1 (R1), Root 2 (R2), Root 3 (R3), Stalk 1 (S1), Stalk 2 (S2), and Stalk 3 (S3).

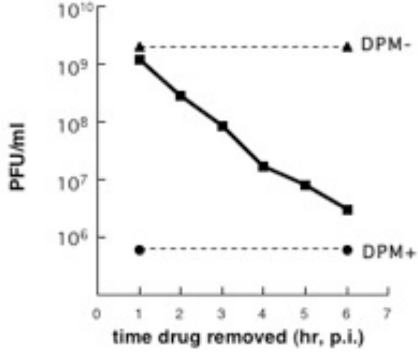

Figure 2. DPM and Virus Yield. HeLa cell monolayers were infected with Mengovirus at a multiplicity of 50 pfu/cell. DPM+ samples had 80 µM DPM (in ethanol) added to the media at the time of infection. DPM- samples were dosed with an equivalent volume of ethanol. Medium from DPM+ cultures was exchanged with drug-free medium at the indicated times. Virus was harvested at 8 hrs PI and the titer determined by plaque assay.

Tables

While Figures are often used in papers (graphs, photographs, gel images) tables are rare. Tables should only be used when all the data being presented cannot be reported in a simple and comprehensible manner in the Results section. The title appears at the top of the table; there is no legend. A footnote may be necessary to clarify an important point in the table.

Table 2. Mengovirus plaque phenotypes in the presence of DPM.

Concentration, μM DPM	Plaque Reduction (%)[a]	Relative Plaque Size[b]
80	100	N/A
60	98	minute
40	93	+
20	68	++
10	25	++
0	0	++++

[a] Values represent the average of two experiments each done in triplicate.

[b] Plaques in the absence of DPM averaged about 2mm in diameter.

Table 1. DNA glycosylases in human cell nuclei.

Enzyme	Size (amino acid residues)	Gene location at chromosome	Altered base removed from DNA
UNG	313	12q23–q24	U and 5-hydroxyuracil
TDG	410	12q24.1	U or T opposite G, ethenocytosine
hSMUG1	270	12q13.1–q14	U (preferentially from single-strand DNA)
MBD4	580	3q21	U or T opposite G at CpG sequences
hOGG1	345	3p25	8-oxo G opposite C, formamidopyrimidine
MYH	521	1p32.1–p34.3	A opposite 8-oxo G
hNTH1	312	16p13.2–p13.3	Thymine glycol, cytosine glycol, dihydrouracil, formamidopyrimidine
MPG	293	16p (near telomere)	3-MeA, ethenoadenine, hypoxanthine

Reference Citation Formatting

A References section lists only those references cited in the paper. You will cite all the references you used when you wrote your paper. In the text of the paper, cite the references using the author's name and publication year. If there are two authors you must state both of their names if there are greater than two authors, state the first authors name followed by et al.

For example: We predict that the 95% alcohol solution will kill all of the NIH-3T3 cells in our culture flask because Smith performed a similar experiment in his paper with another cell type (Smith et al, 1998). In previous research the p58 protein was associated with increased cell growth in C127 cells (Johnson, 2001; Benenson and Kortemeyer, 2003; Haenisch et al, 2006).

Types of Literature:

The vast collection of scientific literature can be generally divided into three categories based on how 'close' they are to the original experiments and descriptions of scientific phenomena. **1) Primary literature**: The bulk of scientific journal articles are primary, meaning that they report the findings of specific experiments or descriptive studies. **2) Secondary literature**: From time-to-time investigators write review articles or books that summarize what is and is not known about a particular topic. Rather than conducting new experiments, these authors rely heavily on the primary literature, therefore these review articles and books are considered a part of the secondary literature. **3) Tertiary literature**: More general texts that summarize what has been reported in review articles comprise the tertiary literature.

Most new research relies heavily on previous work reported in primary literature. However, review articles can be extremely helpful in understanding how your research project fits into the larger scope of scientific investigation, and can be used as a source to locate primary literature references for the topic of interest.

Note that websites were not included in the above description of scientific literature sources. This is because they are not refereed — that is, just about anyone can publish something on the web without some impartial reader reviewing it beforehand. Web pages are often wonderful sources of information, but they can just as often be replete with bad information. At this point, it is very difficult to determine the reliability of web sources and, in general, they should generally only be used as a starting point about a particular topic. ***Thus websites are only allowed as citations in DRAFT1 manuscripts.***

Examples of Proper Citation Formatting for the listings in your Reference section:

Journal articles:

Single Author:

Belsky, A. J. 1986. Does herbivory benefit plants? A review of the evidence. American Naturalist 127: 870–892.

Two Authors:

Brown, J. H. and D. W. Davidson. 1977. Competition between seed-eating rodents and ants in desert ecosystems. Science 196: 880–882.

Multiple Authors:

Free, C. A., J. R. Beddington, and J. H. Lawton. 1977. On the inadequacy of simple models of mutual interference for parasitism and predation. Journal of Animal Ecology 46: 543–554.

If the source was published in an online journal do not cite the URL, treat it the same as printed:

Thomas, J. A., M. G. Telfer, D. B. Roy, C. D. Preston, J. J. D. Greenwood, J. Asher, R. Fox, R. T. Clarke, and J. H. Lawton. 2004. Comparative losses of British butterflies, birds, and plants and the global extinction crisis. Science 303: 1879-1881

Books:

Chapter within a book:

Goldberg, D. E. 1990. Components of resource competition in plant communities. Pp. 27-50 in J. B. Grace and D. Tilman, eds., Perspectives on Plant Competition. Academic Press, San Diego.

An entire book:

Hynes, H. B. N. 1970. The Ecology of Running Waters. University of Toronto Press, Toronto.

Theses:

Watson, D. 1987. Aspects of the population ecology of Senecio vulgaris L. Ph.D. thesis, University of Liverpool.

The textbook:

Campbell NA. and Reese JB. 2007. Biology – 8th ed., Chapter 13 "Mendel and the Gene". Benjamin Cummings, CA.

The Lab Manual:

Igert, et al. 2021. LB144 Course Pack. MSU Printing Services, East Fee Hall. Michigan State University, East Lansing, MI

A Web Site: [only allowed in DRAFT1 manuscripts and follows a text citation (Author(s), Year published).]

Anonymous. 2002. Wisconsin Fast Plants Web Site. http://www.fastplants.org /Introduction/ Introduction.htm, last accessed 7/10/02

References (used in the creation of this appendix):

McMillian, V.E. 2001. Writing Papers in the Biological Sciences, 3rd ed. St. Martin's Press, Inc., New York.

Appendix

Note everything you place in your appendix will be discarded during grading.

An Appendix section includes only materials that are not actually required in the paper and could be thrown away with no effect to the paper. Often special extra information is included here. In LB-144 you are expected to place any laboratory notebook pages that indicated signed data for all members (photocopies from your notebook) and a photocopy of the first page of any articles cited and referenced in the report.

Reminders

Before writing your paper, refer to the following hints to make your paper stronger:

1. Write clearly in short, logical, but not choppy sentences.
2. Use past tense in the Abstract, Methods, and Results sections when discussing things that have been completed. Also uuse past tense in the Introduction and Discussion sections when referring to your experiment. Use future tense when making predictions about future experiments.
3. Write in grammatically correct English, but use METRIC UNITS.
4. When referring to the scientific name of an organism, the genus and species should be italicized, the first letter of the genus is capitalized, but the species is in lower case; for example *Drosophila melanogaster*.

Making a Website

Making a webpage is a useful skill to have and is not as hard as you might think. This primer is intended as a walkthrough for the basic beginner. Anyone looking for a more advanced explanation should enroll in a web design course already.

There are many "places" on the internet where you can load your webpage (each website name corresponds to a file on a computer somewhere in the world) but we'd like you to load your file onto MSU's computers through their AFS system. For the uninitiated, the basic idea of this system is that MSU has provided hard-drive space that users (students and faculty) can access and use from any computer connected to the internet. Most people use this to store and back-up files or run webpages. If you'd like more info on what this system is and how it works, go to the ATS helpdesk website at help.msu.edu.

The first step is to generate your file and save it as an html. There are many programs that can do this for you, but most versions of MS Word made in the last decade have a "save as webpage" or "save as html" function in the file menu. You should title your document "index" (you need at least one index file in the Web folder for MSU to be able to run your web page, so this might as well be it), unless you would like to make another index file that has hyperlinks to the document you are currently trying to upload. If you have images in your file (which you really should have) then Word will automatically make a separate folder titled "index_files" in which all of the images are stored. You will need to create a folder with the same name in your AFS space and upload all of these files into it.

Go to netfiles.msu.edu and log in with your MSU netID. Click on the "Web" folder which should be near the bottom of the file/folder list in the middle of the page. Select "Upload file(s)" from the options on the left. Click on the "Browse"" button, locate your index.htm file and click "Upload file(s)". To create your image folder, click on the "Create a new folder" option on the left side of the screen and enter the name EXACTLY the same as the one generated by Word (index_files). Click on the folder and repeat the file uploading process until of the folder"s contents are in your AFS space.

Now test your website by going to www.msu.edu/~yournetID. If you are having problems getting things to work, contact the ATS help-desk or a resident computer whiz.

Samples: Student Papers

Genotypic Identification of CF Patients with the R553X Mutation using IB3 and S9 Cell Lines and Allele Specific PCR

By: Sarah Bonczyk, Nathan Johns, Elizabeth LeMieux, and Mitch Wood

LB 145 Cell and Molecular Biology
Tuesday 7 PM
Ashley Coulter and Jason Mashni
4/24/2009

http://teamhouselb145.tripod.com/

(Title page written by: Nathan Johns
Revised by: Mitch Wood,
Finalized by: Liz LeMieux)

Abstract

Written by: Nathan Johns
Revised by: Mitch Wood
Finalized by: Liz LeMieux

The R553X mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) accounts for 0.7% of all cystic fibrosis cases (Hull *et al,* 1993). The mutation involves a single base pair substitution in the 553rd amino acid from CGA (Arginine) to TGA, a stop codon, leading to a truncated protein (Bal *et al,* 1991). Allele specific polymerase chain reaction (ASPCR) was used to determine whether samples of DNA are wild-type, heterozygous, or homozygous for the R553X mutation in the CFTR gene. DNA was extracted from epithelial bronchial cells of known CF patients (Qiagen Inc, 2007). We hypothesized that by controlling annealing temperatures and salt concentrations in the PCR reaction, a single base pair mismatch can be used to determine the presence of the R553X mutation, based on previous PCR diagnostic testing (Chavanas *et al,* 1996). The resulting amplified DNA was then analyzed using agarose gel electrophoresis to determine the genotype of the DNA. We were able to determine whether samples were heterozygous, homozygous wild-type, or homozygous for the mutation by the presence or absence of bands 1,056 base pairs long and based on which forward primer used (Wu *et al*, 1989). Research surveys were distributed and analyzed in order to examine different public opinion on genetic testing between students of various studies at Michigan State University. Results showed no significant difference in opinions on genetic testing among various residential colleges. These tests are significant in helping doctors diagnose cystic fibrosis patients for specific mutations faster and more accurately than previous testing.

Introduction

Written by: Nathan Johns
Revised by: Mitch Wood
Finalized by: Liz LeMieux

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The role of the CFTR protein is to serve as a chloride ion channel in epithelial cells (Rowe *et al*, 2008). Epithelial cells with a CFTR mutation, most often in the lungs, pancreas, and intestines, secrete large amounts of mucus, which builds up and creates complications in the affected tissues (Welsh and Smith, 1995). Mucus buildup in the respiratory tract often leads to pulmonary infection, the most common cause of death in CF patients (Golshahi *et al,* 2008). Current treatments attempt to remove this mucus in the lungs and avoid affection, often by using percussive therapy and antibiotics (Welsh and Smith, 1995).

Over one thousand mutations in the CFTR gene are identified to cause CF, with the ΔF508 mutation, a deletion of three base pairs at position 508, being the most common, and accounting for approximately 70% of all cases (Teem *et al*, 1993). The R553X mutation is the sixth most common, accounts for 0.7% of cases, being most prevalent in German communities (Hull *et al*, 1993). R553X is a nonsense mutation caused by a C to T substitution at the 553rd amino acid. This changes what would normally be arginine to a stop codon (Hull *et al,* 1993). A nonsense mutation is a mutation that causes the DNA sequence to result in a premature stop codon, or a nonsense codon in the mRNA which results in a truncated, incomplete and nonfunctioning protein. In the case of R553X, research has shown that the premature stop codon often results in exon skipping in RNA translation (Aznarez *et al*, 2007). The loss of the exon causes an unstable mRNA of the truncated protein and therefore does not undergo the process of translation (Aznarez *et al*, 2007). The R553X mutation is a class I mutation because of the unstable mRNA synthesized in the nucleus caused by nonsense alleles. (Gambardella *et al.* 2006)

Polymerase chain reaction (PCR) is a technique used to amplify a desired section of DNA (Saiki *et al*, 1988). In PCR, DNA is heated during a denaturing step in order to break the hydrogen bonds between nucleotide bases to separate complimentary 5’ and 3’ strands. Once

Methods

Written by: Liz LeMieux
Revised by: Sarah Bonczyk
Finalized by: Mitch Wood

Primers

Before the PCR tests were run, primers for the PCR tests were designed using the Cystic Fibrosis Mutation Database. Forward and reverse allele-specific primers that worked with both the wild type and with the mutation were needed. FPrimer1 is a forward primer ending in Guanine, complementary to the wild type amino acid where the mutation should be present. The second of the forward primers, Fprimer2, seeks the mutant type base sequence at the mutation site, base pair number 1789, which results in the primer ending in Adenine. The reverse primer, Rprimer, was designed to bind to the DNA strand 1,022 base pairs past the mutation site between base pairs 2828 and 2811 and was used in both tests. Fprimer1 is 16 base pairs long with the sequence of: GACTCACCTCCAGTT**G** and should properly bind to the wild type gene sequence of **C**AACTGGAGGTGAGTC. Fprimer2, the mutant seeking primer, is also 16 base pairs long with the sequence of: GACTCACCTCCAGTT**A**; the only difference from the previous forward primer is the last base pair, which should properly bind to the mutant R553X gene sequence of **T**AACTGGAGGTGAGTC. The reverse primer that will be used for both tests is 18 base pairs long with the sequence of: CATGAGAGAAGAGAC, which should bind to the gene sequence of TCTGTCTCTTCTCTCATG, which is the same in both mutant and wild type genes. All of the primers are written in 5' to 3'. These primers were ordered from the biological laboratory company Integrated DNA Technologies (IDT).

After the primers were designed, the annealing temperatures at which the primers would bind were determined by first calculating the theoretical melting temperatures of each primer using the following formula:

$$Tm=64.9° C + 41° C \times (\text{number of G's and C's in the primer} - 16.4)/N$$

where N is the length of the primer (Wright *et al,* 2009). The calculated theoretical melting temperatures were: Fprimer1- 45.94°C, Fprimer2 – 43.36°C, and Rprimer – 45.77°C. Based on these calculations, an annealing temperature of 42°C was used in the PCR tests.

DNA Purification

DNA Purification was used to collect DNA from cultured cells of both mutant type and wild type samples to be used in PCR. Two different sources of Human DNA came from Human bronchial epithelial cells from a CF patient without the R553X mutation (IB3 stock cells) while another set of stock cells was found to obtain the R553X mutation, therefore were used as the mutant type test (S9 cells). Each of these stock cells were used to contrast the effectiveness of the allele specific primers. (Gambardella *et al*, 2006) Before beginning purification, all reaction vessels were kept on ice until used and frozen cultured cells were allowed to thaw in a water bath set to 37°C. Then, 200ul of the sample (either human bronchial epithelial cells without the mutation or cells from a CF patient with the mutation, depending on the PCR test being done) was added to a capture column and was incubated at room temperature for 30 minutes. Afterwards, 400ul of DNA Purification Solution 1 was added to the sample. The resulting mixture was incubated for 1 minute at room temperature. After incubation, the mixture was centrifuged for ten seconds at 2,000-12,000 times the force of gravity (xg). The capture column was then transferred into a new waste collection tube. Another 400ul of DNA Purification Solution 1 was added to the mixture and the solution was incubated at room temperature for 1 minute. The solution was then centrifuged again for ten seconds at 2,000-12,000 x g. Then, 200ul of DNA Elution Solution 2 was added and the mixture was centrifuged for ten seconds at 2,000-12,000 x g. The capture column was transferred to a clear DNA collection cube and 100ul of DNA Elution Solution 2 was added. The solution was incubated for ten minutes at 99°C and then centrifuged for 20 seconds at 2,000-12,000 x g. (Quigen Inc. 2007)

PCR

To analyze DNA obtained from the purification process, two different Polymerase Chain Reaction (PCR) tests were designed. Test #1used Fprimer1 and the Rprimer. Test #2 used Fprimer2 and the Rprimer. For each of the tests, the combination of 2.0ul of the target DNA template obtained through DNA purification, 5.0ul 10X PCR buffer, 0.2ul Taq polymerase, 2.0ul forward primer (100 uM), 2.0ul reverse primer (100 uM), 1.0ul 10mM deoxynucleotide building blocks (dNTP) of DNA and 40.4ul water was added to a test tube on ice. The combination was mixed and spun down in a centrifuge. Once the sample was placed in the Labnet thermocycler, the cocktail went through five steps: initial denaturation, denaturation, primer annealing, extension and final extension. In the initial denaturation stage the temperature was raised to 94°

Predicted Results

Authored by: Kevin Werner

In this experiment for the template used in PCR, genomic DNA will be extracted and purified from human cells. Cultured cells from Crohn's Disease patients with the R702W-CARD15 mutation, without the mutation, and heterozygous for the mutation will be obtained from CRISI Inc (Sacramento, CA). For our diagnostic assay, PCR will be used to amplify a DNA segment at a locus containing the R702W mutation on the CARD15 gene that causes a form of Crohn's Disease using a specialized approach for detecting SNPs developed by Hidenobu Yaku (Yaku et al, 2008). The resulting PCR products will be analyzed using agarose gel electrophoresis in either a traditional TBE or fast LB buffer system.

Primer Design

We predict PCR of the genomic DNA will amplify the anticipated products from the R702W locus on the CARD15 gene on chromosome 16 (Figure 1). This will occur because of carefully selected primer design, reactant concentrations, and PCR temperatures and times (Saiki et al, 1998). Thus primers R (annealing at 20,866 base pairs to 20,887 base pairs on the CARD15 gene) and CDF (annealing at 19,866 base pairs to 19,881 pairs on the CARD 15 gene) will amplify a 1021 base pair product, and primers R and WTF (annealing at 19,866 base pairs to 19,881 base pairs on the CARD15 gene) will amplify a 1021 base pair product (Wright, et al. 2010) (Figure 2). Also, the forward primers CDF (forward primer designed to anneal to mutant type template) or WTF (forward primer designed to anneal to wild type template) will not anneal when their

bases are not completly complimentary to the DNA template bases at the respective annealing loci of the primers, preventing amplification from occurring (Schochetman et al, 1988). The forward primers are known as discriminating primers because they are designed to be completely complimentary to either the mutant type or wild type genotype, but not both (Wittwer et al, 1993). Hence it is also predicted that heterozygous DNA will allow for some amplification with both of the discriminating primers. It then follows that amplification of DNA template from the CDF discriminating primer supports the presence of the R702W mutation, which could cause a maladaptive pro-inflammatory response in Paneth cells along with other genetic and environmental factors (Figure 3) (Lala et al, 2003).

We did a preliminary PCR amplification of the 1542 base pair long 16S rDNA locus of *Escherichia coli* to use as a control throughout our experiment (Haffar et al, 2010). The bands we produced during gel electrophoresis exhibited non-specific binding that we were able to reduce by raising the annealing temperature (Figure 4) (Livak et al, 2010). Also, the validity our concentrations of forward primer, reverse primer, dNTPs, buffer, and taq polymerase will supported by these experiments, thus supporting the validity of our Crohn's experiment.

Genomic Purification

In our preliminary research and assay design we found that DNA is extracted from samples in four steps: cell lysis, membrane disruption with a detergent, protein removal, and precipitation (Qiagen 2010) (Figure 5). We predict DNA yield of purified samples will be in the range of 3-8 μg, because this is the normal theoretical yield of

understanding of issues like genetic testing significantly more than any other surveyed group (Figure 6). Using the 1-10 scale for all questions pertaining to genetic testing, all surveyed groups on average responded above the median answer of 5, from which it can be interpreted that Michigan State University students tend to be supportive and knowledgeable about genetics (Table 2).

Discussion

Written by: Mitchell Wood
Revised by: Nathan Johns
Finalized by: Sarah Bonczyk

Experiment Summary

Cystic fibrosis, the most common autosomal recessive disease in Caucasians (Aznarez *et al*, 2007), has dramatic effects on multiple organs, including the lungs, pancreas, intestines, and liver (Welsh and Smith, 1995), due to mutations of the CFTR gene on chromosome seven, causing defects in sodium and chloride transport in epithelial cells (Aznarez *et al*, 2007). The R553X mutation is a specific variation of cystic fibrosis, involving a single base pair substitution at the 1789th base pair in the 553rd amino acid, from cytosine to thymine (Hull *et al*, 1993). The subsequent change from the amino acid arginine to a premature stop codon causes early truncation of the CFTR protein, thus altering the folding sequence (Gambardella *et al.* 2006). Although PCR has been proven effective for diagnosing genetic disorders such as cystic fibrosis (O'Leary *et al*, 1997), the question we are addressing is whether or not a PCR test can be designed to identify this specific mutation. We hypothesized that allele specific primers and a single base pair mismatch could be used to develop an accurate diagnostic test for patients with the R553X mutation using experimentally determined optimal conditions of PCR in terms of annealing temperature, primer concentration, and salt concentration.

In addition to primer design, we bridged the gap between laboratory experiments and the sociology behind diagnosing genetic diseases by surveying student opinion on genetic screening and the effects of genetic diseases on the human race in the long run. In recent years modern medicine has extended the life expectancy of people with cystic fibrosis allowing those affected to live to childbearing age (Ratjen 2008), meaning CF genes are more likely to be passed down to future generations. Samples were taken from Lyman Briggs, James Madison, the College of

Natural Science, and general university students. It was hypothesized that Lyman Briggs students would be more supportive of genetic testing due in part to their background in required integrated studies and their greater understanding of how the frequency of a genetic disease could impact a gene pool (Singer *et al*, 2008).

Original Predictions

By amplifying DNA from IB3 human bronchial epithelial cells from a CF patient and S9 epithelial cells from a leukemia patient via allele specific PCR, the length of the amplified DNA was interpreted through gel electrophoresis to show the presence or absence of the R553X mutation. Two different forward primers, Fprimer1 and Fprimer2, were designed to discriminate between the wild-type and mutant CFTR genes through allele specificity based on a single base pair mismatch on the 3' end. The mismatch was positioned on the 3' end of the primers to more effectively reduce the amplified product by decreasing DNA polymerase and dNTP binding efficiency (Yaku *et al*, 2008). Successful annealing of the primers and the subsequent extension phase was hypothesized to result in a band of 1,056 base pairs, thus indicating a positive test. The lack of a band was hypothesized to indicate a disruption in the extension phase due to the single base pair mismatch (Chavanas *et al*, 1996). A homozygous wild-type genotype was expected to show a band of 1,056 base pairs when using Fprimer1 and show no band when using Fprimer2. In contrast, a homozygous mutant genotype was expected to show a band of 1,056 base pairs when using Fprimer2 and show no band when using Fprimer1. Lastly for heterozygous genotypes, faint bands 1,056 base pairs long were expected to appear in both tests, using either Fprimer1 or Fprimer2 due to the replication of both genotypes during PCR, causing neither set of forward primers to completely discriminate against the specific mutation site (Chavanas *et al*, 1996).

Results and Ultimate Findings

In order to determine optimal PCR conditions, multiple experimental trials were run with adjustments in DNA concentration and primer concentration. In addition, multiple experiments were run to establish the optimal annealing temperature, which can directly affect the annealing rates of designed primers (Elnifro *et al*, 2000) and magnesium chloride ($MgCl_2$) concentration to alter magnesium ion concentrations, which directly affect DNA polymerase activity in PCR (Ignatov *et al*, 2002). The optimal annealing temperature was determined to be 46ºC based off of the calculated primer melting temperatures (see Methods section) and which annealing

References

Written by: Mitchell Wood
Revised by: Nathan Johns
Finalized by: Sarah Bonczyk

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Predicted Figures:
Authored by: Kevin Werner

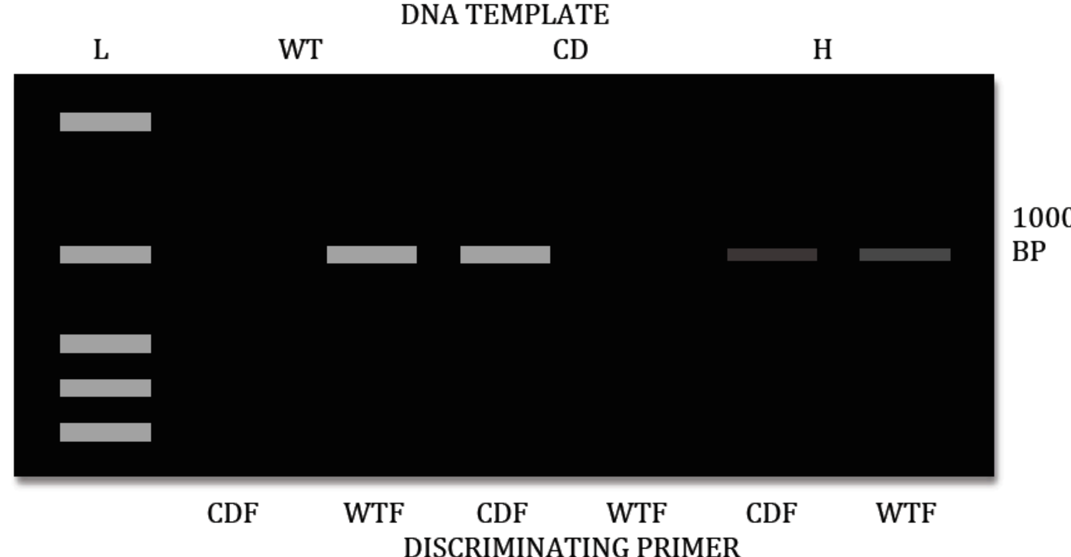

Figure 1 – Predicted results of from amplified products using gel electrophoresis. The non-discriminating oligonucleotide reverse primer, R, is 2% 3'-GTGGCTGCAGGGTTACAACTA-5'. The discriminating oligonucleotide forward primer CDF is 2% 5'-CGGGACGAGGCCGCG-3'. The discriminating oligonucleotide forward primer WTF is 2% 5'-GCGGGACGAGACCGCG-3'. The predicted binding site is on chromosome 16 on the CARD 15 gene from position 19,866 b.p. to 19,881 b.p. for the forward primer CDF, from position 19,866 b.p. to 19,881 b.p. for the forward primer WTF, and from position 20,866 b.p. to 20,887 b.p. for the reverse primer R with respect to the CARD 15 gene. It is predicted there will be a yield of 1.7 billion copies of the target DNA, along with a negligible amount of much larger DNA fragments (a by-product of PCR), and the original DNA template. The denaturing temperature will be set at 95°C, the annealing temperature at 55°C, and the extension temperature at 72°C. An initial denaturing time of two minutes will be used, then the times will be 30 seconds denaturing, 45 seconds annealing, one minute extending, and a final extension of 7 minutes for 30 cycles. The agarose gel contains 10% agarose. All bands are predicted to appear at 1021 base pairs beside the ladder. Bromphenol blue dye will be added to the gel to indicate when the gel electrophoresis is complete. L is the molecular marker or ladder, and the DNA templates WT, CD, and H are 2% DNA purified products from a wild type, mutant, and heterozygous genotype, respectively.

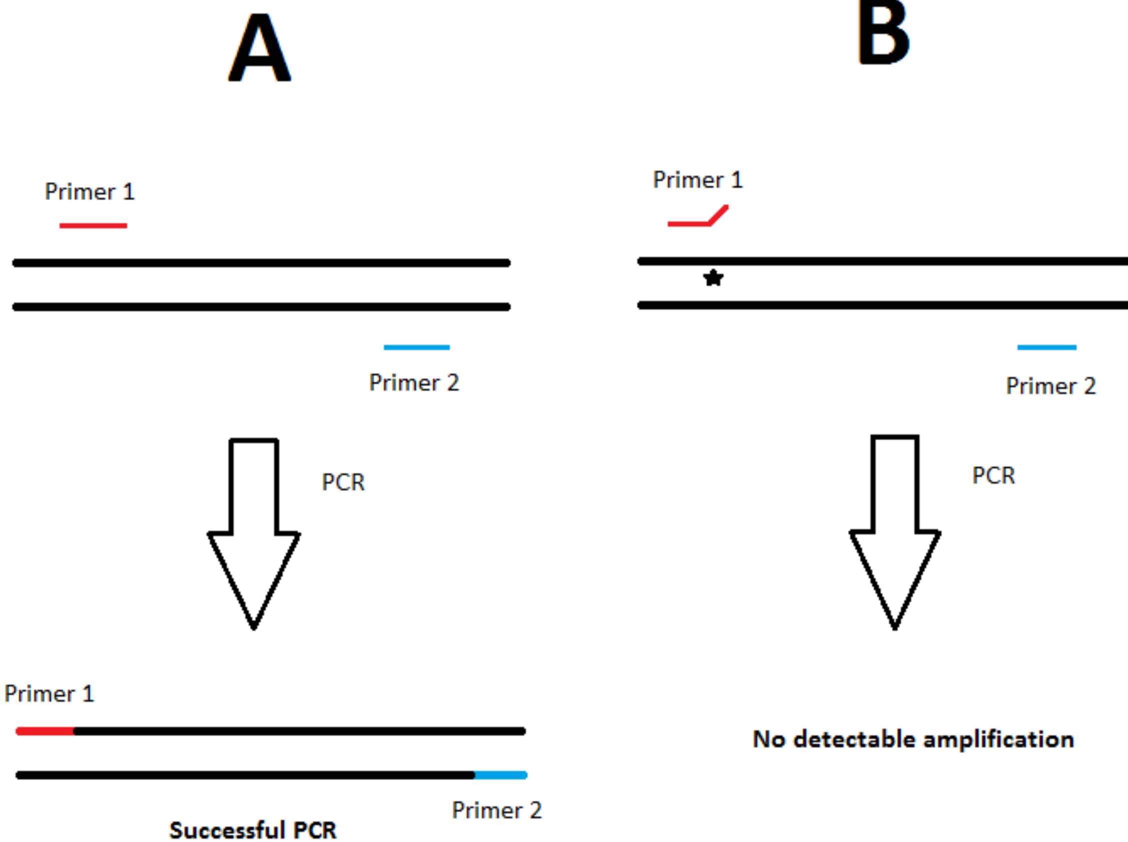

Figure 8. Representation of allele specific PCR. Column A represents wild-type DNA that was extracted using the "generation capture column kit". Column B represents mutant DNA. Primer 1 represents the forward wild-type primer and primer 2 represents the reverse primer. When primer 1 is used with wild-type DNA as seen in column A, PCR completes successfully. This is because the oligonucleotide bases match up with the DNA template and primers. However, when we use primer 1 with mutant DNA as shown in column B, the result is that there is no detectable amplification of DNA.

Figures

Written by: Sarah Bonczyk
Revised by: Liz LeMieux
Finalized by: Nathan Johns

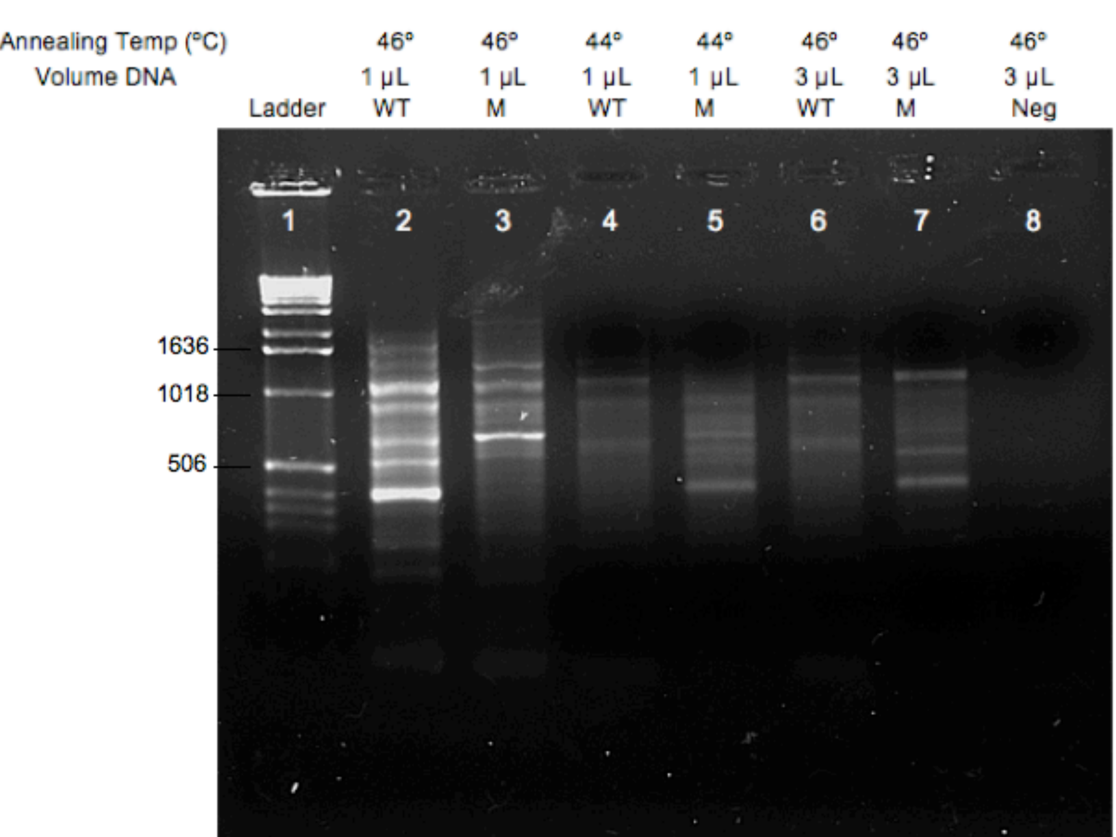

Figure 1: Amplification of DNA segment containing R553X mutation site while varying annealing temperatures and initial S9 DNA volumes. Thermocycling conditions included a 5-minute denaturation at 94 ºC with 30 cycles of 30 s at 94 ºC, 30 s at 46 or 44 ºC, and 60 s at 72 ºC, with a final elongation phase at 72 ºC for 7 minutes. All lanes show non-specific binding. Lanes 2,3,4,6, and 7 show bands near 1018. The targeted region of DNA is 1056 base pairs in length. Lanes using an annealing temperature of 46 ºC and lower initial DNA volumes of 1µL show higher intensity bands than other lanes. From this test it was determined that lower initial concentrations of DNA and an annealing temperature of 46 ºC are optimal for our designed primers.

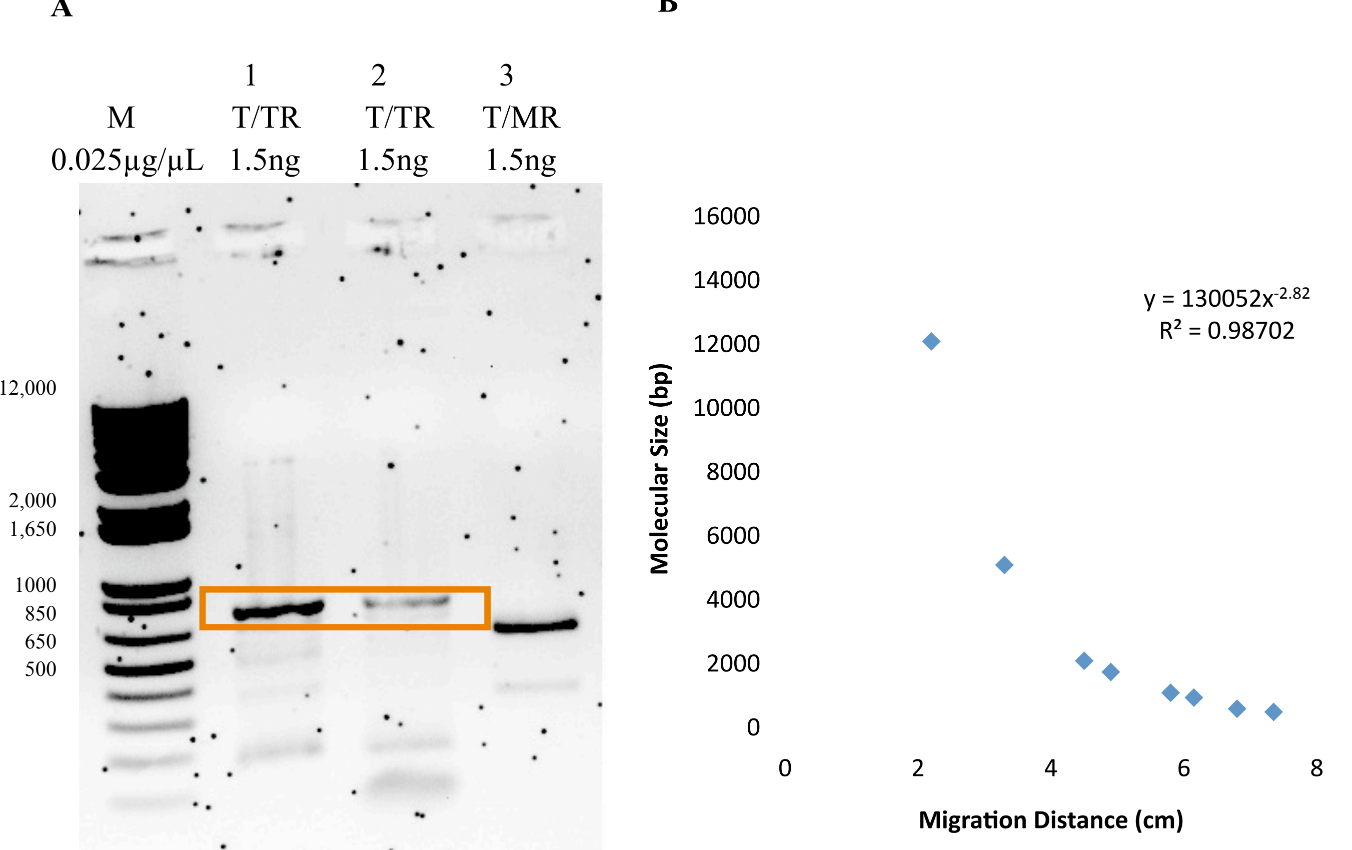

Figure 2. PCR amplification of the DMD gene from human DNA at an annealing temperature of 48ºC. A. After PCR amplification, gel electrophoresis was conducted in a 0.8% TBE gel run at 115V for 30 minutes to detect amplified regions of DNA. M is the molecular marker (1.25ng) 1-Kb Plus Ladder. Lanes 1 and 2 correspond to wild-type DNA samples tested with the wild-type forward/reverse primer set (T/TR). 10µL (1.5ng) of wild-type DNA was added to each of these lanes, and the expected amplification of a 765bp long fragment was observed. Lane 3 corresponds to a wild-type DNA sample amplified with the mutant forward/reverse primer set (T/MR). 10µL (1.5ng) of DNA was added to this lane and a PCR product of 589bp long was observed. For each one of the lanes, non-specific binding was also seen but the expected band was quite distinct.

B. Semi-log plot for 1.25ng of 1-Kb Plus ladder. Each point of the graph depicts the specific distance traveled by each band of the 1-Kb Plus ladder with respect to its well. An R^2 value of 0.98702 ($p<0.05$) was obtained for the logarithmic trend line of the plotted traveled distances. The equation obtained from the trend line was used to calculate the traveled distances of the bands from lanes 1, 2, and 3 to get more accurate band size values. Values of 757.83 ± 8.75bp, 775.33 ± 8.75bp and 589.03 ± 5.21bp were obtained for lanes 1, 2, and 3 respectively.

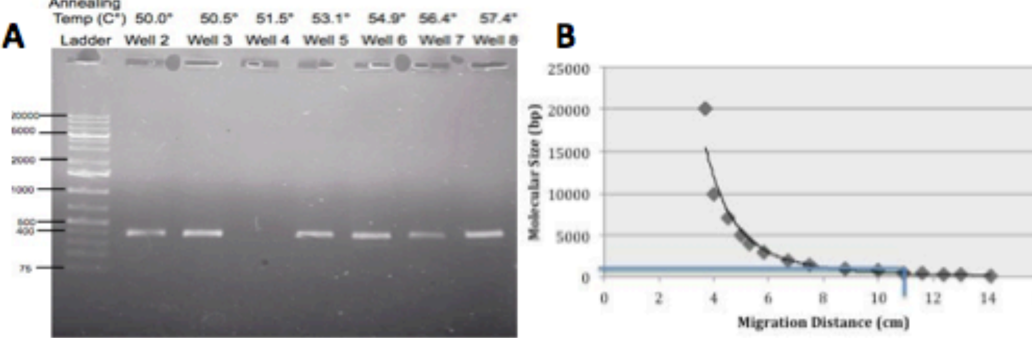

Figure 3: Amplification of lambda RZ gene by PCR and analysis by gel electrophoresis. **(A)** A target DNA sequence of about 400 bp was amplified using PCR in which two primers were used: Rz1R and Rz1F. The PCR cocktails contained 38 µL of nuclease free water, 7 µL of 10X PCR buffer, 1 µL of Taq polymerase, 1µL of dNTPs, 1µL of lambda DNA template, and 1µL of each primer. The cocktails were run in the thermocycler at 95° C for an initial 3 minutes and then cycled between 30 seconds at 95° for the denaturing phase, 30 seconds at the annealing temperature for the annealing phase, and 1 minute at 72° for the elongation phase. The annealing temperatures were set on a gradient from 50° to 58° and 25 cycles were completed. Annealing temperatures are noted above the wells. A 0.8% agarose gel made using TBE (Tris/Borate/EDTA) buffer, agarose, and GloGreen was run at 135V in which 7µL of DNA and 3 µL of loading dye was pipetted into wells 2-8 and 5µL of Fermentas' 1kb Plus ladder was pipetted into well 1. The gel was then observed under an ultraviolet light in order to see the bands created by the DNA product. **(B)** Migration distance vs. molecular size of 1 kb Plus DNA ladder used to analyze PCR products from Rz gene. A target sequence of lambda DNA from the Rz gene was amplified using PCR. An 0.8% agarose gel was made using TBE (Tris/Borate/EDTA) buffer, agarose, and GloGreen. 5 uL of 1 kb Plus ladder was used and analyzed using a semi-log plot shown above in which the x-values represent the distance in centimeters that the bands of the ladder migrated away from the well and the y-values represent the size of the molecules in base pairs. A trend line was added in order to obtain an equation for further analysis. This equation was used to calculate the base pair length of the lambda DNA PCR product in which the distance migrated from the wells was inputted for the x-value of the equation, yielding a y-value representing the base pair length of the product. For the bands produced by the lambda DNA (Figure 5), the base pair length produced was calculated to be 400 bp for all 6 wells yielding a band. The R2 value given in the figure represents the fit of the trend line; 1 represents a perfect fit.

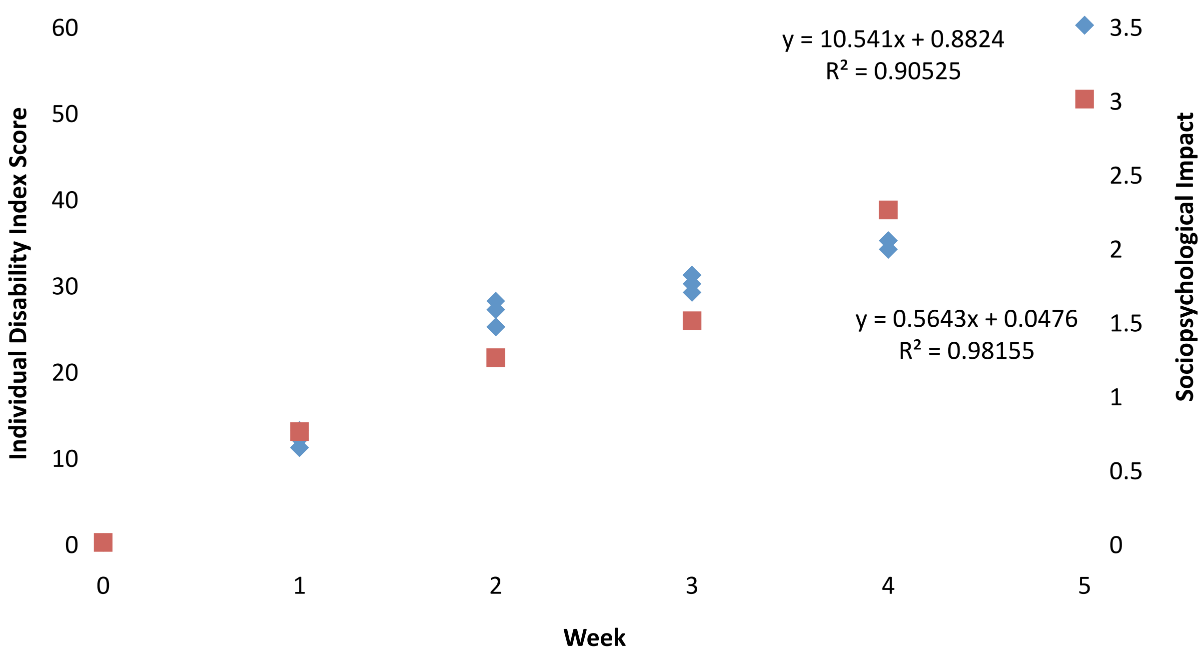

Figure 5. Correlation between the degenerative nature of Duchenne muscular dystrophy and the deteriorating socio-psychological state associated with the disease. Duchenne muscular dystrophy's symptoms were progressively added to each one of the researchers' lifestyles every week for a five-week period. At the end of each week, each researcher completed the Disability Index of Stanford University's Health Assessment Questionnaire (HAQ) in order to gauge the severity of disability that the researchers were living with. The HAQ gives scores between 0 and 60, with zero pertaining to no disability at all and sixty symbolizing complete disability and immobility. In addition to the HAQ, each researcher completed a survey that measured, on a scale of 0 to 3, the socio-psychological impact on the researcher as each symptom was added. A linear regression for the individual Disability Index scores is shown in blue while the linear regression for the Socio-psychological impact test scores is shown in red. An R^2 value of 0.90525 ($p<0.05$) was obtained for the Disability Index score and an R^2 value of 0.98155 ($p<0.05$) was obtained for the socio-psychological impact test.

Table 1. Chi-square test on adverse reactions to CF symptoms in public locations.

Location	Total People Observed	Expected Frequency*	People Observed with Reaction	People Expected with Reaction	$(O-E)^2/E$
Elevator	40	.717	27	28.68	.0984
Cafeteria	25	.717	17	17.93	.0482
Study Lounge	15	.717	9	10.75	.284
Restaurant	10	.717	6	7.17	.191
Total	**90**	**.717**	**59**	**64.53**	X^2=.6216
				n=4	df=3
					p value associated with X^2=.90

*Values representative of the results of question one of Figure 5.

Grading Rubrics

The "Proposal"

What will I have to do?

You will write a title and 2 paragraphs for your group's Proposal (an Introduction & Methods paragraph).

The "Proposal" paper from a group-of-4 will include: A title page with four titles, an Introduction with four paragraphs, and a Methods with four sections. Each student should put their B-PID by their writing so <u>your</u> Title, Intro, Methods can be <u>your</u> grade.

Each student’s grade is based 100% on their sections’ score. The relevant sections on the DRAFT1 rubric (provided in your Course Pack) will then be used to grade your group's Proposal.

To review, each student will be responsible for authoring:
1. Their own title
2. Their own paragraph (with numerous citations) in the Introduction
3. Their own paragraph/section of the Methods
(we recommend you create a References section, but that is not graded)

TIPS: Your group must coordinate what each person writes about so the final Proposal paper is logical and professional (e.g. each paragraph of the Introduction must discuss different topics). For example: if the first paragraph of the Introduction discusses why beavers are an important part of the economy of Minnesota, the second might then be on a certain communication behavior that is used, the third could be about a Method used to study that behavior and the fourth on a gene that may be important for the animal to perform that behavior. Similar approach in the Methods section, each student should write a paragraph that explains a different Method or part of a method that will be used in your research.

Good news: For the Proposal, your grade is entirely based upon *your* sections! If one of the students in your group never does any of their work, and provides no writing for the final Proposal, it has zero impact on your grade. Just write in pencil on the title page something like “Mr. Hayes had a previous engagement in the Bahamas and sadly was unable to participate in this assignment.”

More good news: Blind grading will be used! Be sure to never indicate anyone’s name on the manuscript (just B-PID numbers) thus when grading occurs each grader's prior interactions or expectations cannot have an influence on the score. For example, grader likes this section because they liked the author (or the opposite).

Group Inquiry Research Paper DRAFT 1
Due at the start of Lab

****REJECT and HAND BACK IMMEDIATELY if…***
__ The individual authorship of sections is unclear.
__ "Group responsibility" signature page is not completed
__ Submitted manuscript format doesn't not follow "Instructions to Authors"
__ Submitted manuscript has not yet been uploaded to http://turnitin.com/ (or have receipt attached)

ORIGINALITY/CREATIVITY/PROFESSIONALISM: (WORTH 2 POINTS *EC*) ____ **ec pts?!**

__ **Appendix: (WORTH 2 POINTS)** Do they have copies of signed data *and* the first page of all references?

Title: (WORTH 3 POINTS)
__ Paper gets 2 pts for having a title that says something about **methods** and their **specimen**.
__ Paper gets 1 points if their title predicts anything about their expected **findings**.

Abstract: (WORTH 5 POINTS)
__ Paper gets 3 points for having an abstract that explains well what they **will do and why.**
Including: ___purpose ___hypothesis ___experimental design ___significance in science
__ 2 points if abstract ***predicts*** anything about their expected **findings/results** *with* support from citations.

Introduction: (WORTH 5 POINTS)
__ Paper gets 4 points for having an introduction that tells you some **background** (several paragraphs) about what they plan to do and (one/final paragraph) what they **expect**/predict to see.
__ Paper gets the final 1 point if their introduction talks about **anyone else's research** on this topic or other related reference information from a book or paper etc.
__ Paper gets 1 BONUS point for having an Introduction that makes you **excited** and want to read on.

Methods: (WORTH 5 POINTS)
__ Paper gets 5 points for having a detailed methods section that clearly tells what they will do in a few pages. If you were an LB-144 student and ONLY had the Methods section, **could you repeat** the work?
Including: ___source of materials ___procedures and data analysis ___narrative & in past tense

"Predicted" Results and Figures: (WORTH 5 POINTS)
__ Paper gets 2 points if "Predicted" Results section discusses expected/predicted data that will be collected and supports assertions with rationale and citation of paper. Is each Figure cited properly, does it make sense?
Including: ___outcomes of experiments predicted with support ("We predict ... because... citation/paper")
__ Paper gets 1 point for each "prototype" figure that seems highly appropriate for their investigation.
Including: ___do Figures address research question ___ have a professional appearance ___have long legends with title and extensive description (so a 144 student could explain it with only that page).

Discussion: (WORTH 5 POINTS)
Try to develop a hypothesis that might be tested in your research. Present **why** you have predicted the results you did? The Discussion should be based on **evidence** found in your citations: books, magazines, literature.
__ 1. has an appropriate and interesting TOPIC for the circumstances;
__ 2. has a clear, and preferably original, specific POINT (also known as a THESIS);
__ 3. provides adequate SUPPORT (REASONING and EVIDENCE) for that point; *requires citations*
__ 4. is well ORGANIZED so that the audience can follow the points and examples;
__ 5. employs CLEAR, PRECISE LANGUAGE;
__ 6. is factually ACCURATE and also FAIR, including recognizing objections; *requires citations*
__ 7. is presented in a way that is ENGAGING to the audience;
__ 8. is of a REASONABLE SIZE for the circumstances (not too short or too long);
__ 9. clearly CREDITS OTHERS when their ideas and words are used; *requires citations*
__ 10. Indicates original PREDICTIONS and ultimate findings

The "Half-DRAFT"

What will I have to do?

You will revise 2 paragraphs from your group's Proposal and create a Results paragraph and corresponding figure.

The "Half-Draft" paper your group submits will include: Title page, Introduction, Methods, Results and Figures sections (only a total of 4 Figures, whether predicted or your data or a mix of both, will be allowed).

So to make your Half-DRAFT your group will revise the Title, Intro and Methods you wrote for the Proposal, and add new sections. Each student's grade is based 75% on their individual sections' score, 25% on the final whole paper score. The relevant sections of DRAFT1 rubric will grade the Half-Draft (pts doubled).

Who is assigned to author which part(s)?

<u>Group of 4:</u>
Group works together to pick favorite TITLE (and then make a title page)

PID- responsible for authoring/revising 1st half of INTRO, creating 1 section of RESULTS and limited/permitted to create only 1 Figure (which should be cited in their RESULTS section).

PEDE- responsible for authoring/revising 2nd half of INTRO, 1 section of RESULTS and limited/permitted to create only 1 Figure (cited in their RESULTS section).

DRG- responsible for authoring/revising 1st half of METHODS, 1 section of RESULTS and limited/permitted to create only 1 Figure (cited in their RESULTS section).

LTP- responsible for authoring/revising 2nd half of METHODS, 1 section of RESULTS and limited/permitted to create only 1 Figure (cited in their RESULTS section).

<u>Group of 3:</u>
Group works together to pick favorite TITLE (and then make a title page)

PEDE- responsible for authoring/revising INTRO, 1 section of RESULTS and limited/permitted to create only 1 Figure (which should be cited in their RESULTS section).

DRG- responsible for authoring/revising 1st half of METHODS, 1 section of RESULTS and limited/permitted to create only 1 Figure (cited in their RESULTS section).

LTP- responsible for authoring/revising 2nd half of METHODS, 1 section of RESULTS and limited/permitted to create only 1 Figure (cited in their RESULTS section).

Group Inquiry Research Paper DRAFT 1
Due at the start of Lab

****REJECT and HAND BACK IMMEDIATELY if…***
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__ "Group responsibility" signature page is not completed
__ Submitted manuscript format doesn't not follow "Instructions to Authors"
__ Submitted manuscript has not yet been uploaded to http://turnitin.com/ (or have receipt attached)

ORIGINALITY/CREATIVITY/PROFESSIONALISM: (WORTH 2 POINTS *EC*) ____ **ec pts?!**

__ **Appendix: (WORTH 2 POINTS)** Do they have copies of signed data *and* the first page of all references?

Title: (WORTH 3 POINTS)
__ Paper gets 2 pts for having a title that says something about **methods** and their **specimen**.
__ Paper gets 1 points if their title predicts anything about their expected **findings**.

Abstract: (WORTH 5 POINTS)
__ Paper gets 3 points for having an abstract that explains well what they **will do and why.**
Including: ___purpose ___hypothesis ___experimental design ___significance in science
__ 2 points if abstract ***predicts*** anything about their expected **findings/results** *with* support from citations.

Introduction: (WORTH 5 POINTS)
__ Paper gets 4 points for having an introduction that tells you some **background** (several paragraphs) about what they plan to do and (one/final paragraph) what they **expect**/predict to see.
__ Paper gets the final 1 point if their introduction talks about **anyone else's research** on this topic or other related reference information from a book or paper etc.
__ Paper gets 1 BONUS point for having an Introduction that makes you **excited** and want to read on.

Methods: (WORTH 5 POINTS)
__ Paper gets 5 points for having a detailed methods section that clearly tells what they will do in a few pages. If you were an LB-144 student and ONLY had the Methods section, **could you repeat** the work?
Including: ___source of materials ___procedures and data analysis ___narrative & in past tense

"Predicted" Results and Figures: (WORTH 5 POINTS)
__ Paper gets 2 points if "Predicted" Results section discusses expected/predicted data that will be collected and supports assertions with rationale and citation of paper. Is each Figure cited properly, does it make sense?
Including: ___outcomes of experiments predicted with support ("We predict ... because... citation/paper")
__ Paper gets 1 point for each "prototype" figure that seems highly appropriate for their investigation.
Including: ___do Figures address research question ___ have a professional appearance ___have long legends with title and extensive description (so a 144 student could explain it with only that page).

Discussion: (WORTH 5 POINTS)
Try to develop a hypothesis that might be tested in your research. Present **why** you have predicted the results you did? The Discussion should be based on **evidence** found in your citations: books, magazines, literature.
__ 1. has an appropriate and interesting TOPIC for the circumstances;
__ 2. has a clear, and preferably original, specific POINT (also known as a THESIS);
__ 3. provides adequate SUPPORT (REASONING and EVIDENCE) for that point; *requires citations*
__ 4. is well ORGANIZED so that the audience can follow the points and examples;
__ 5. employs CLEAR, PRECISE LANGUAGE;
__ 6. is factually ACCURATE and also FAIR, including recognizing objections; *requires citations*
__ 7. is presented in a way that is ENGAGING to the audience;
__ 8. is of a REASONABLE SIZE for the circumstances (not too short or too long);
__ 9. clearly CREDITS OTHERS when their ideas and words are used; *requires citations*
__ 10. Indicates original PREDICTIONS and ultimate findings

The "Draft1" final paper

What will I have to do?
You will take full responsibility for generating a specific section(s) to create your group's final paper.

The "Draft1" paper your group submits will include: Title page, Abstract, Introduction, Methods, Results, Figures, and References sections (in the APPENDIX you just need to provide your graded Half-Draft so we can compare your original version to this one).

Use PIDs not names to indicate who was responsible for each section of the manuscript. But please do *not* keep PIDs associated with each and every paragraph of sections, follow authorship used by the student sample paper in the Course Pack.

Each student's grade is based 50% on their individual sections' score, 50% on the final whole paper score. The relevant sections of DRAFT1 rubric will grade the final paper (pts doubled to 60).

Who is assigned to author which part(s)?

Group of 4:
PID- responsible for authoring/revising all FIGURES.

PEDE- responsible for authoring/revising METHODS.

DRG- responsible for authoring/revising RESULTS

LTP- responsible for authoring/revising TITLE PAGE, INTRODUCTION and REFERENCES.

Group of 3:
PEDE- responsible for authoring/revising METHODS.

DRG- responsible for authoring/revising RESULTS and FIGURES.

LTP- responsible for authoring/revising TITLE PAGE, INTRODUCTION and REFERENCES.

Group of 2:
In a group of 2 one student is the **PID** & **PEDE**, the other is the **DRG** & **LTP**. Hence follow assignments for a group of 4.

Note: Individual responsibilities for the making of your 5-minute-long documentary film are indicated on page where the Roles are described in the Course Pack.

Group Inquiry Research Paper DRAFT 1
Due at the start of Lab

REJECT and HAND BACK IMMEDIATELY if…
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Including: ___source of materials ___procedures and data analysis ___narrative & in past tense

Results and Figures: (WORTH 5 POINTS)
__ Paper gets 2 points if Results section discusses data that was collected and supports assertions with rationale. Is each Figure cited properly, does it make sense?
Including: ___outcomes of experiments clear __ Paper gets up to 1 point for each novel figure that seems appropriate for their investigation.
Including: ___do Figures address research question ___ have a professional appearance ___have long legends with title and extensive description (so a 144 student could explain it with only that page).

Discussion: (WORTH 5 POINTS)
Try to develop a hypothesis that might be tested in your research. Present **why** you have predicted the results you did? The Discussion should be based on **evidence** found in your citations: books, magazines, literature.
__ 1. has an appropriate and interesting TOPIC for the circumstances;
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__ 3. provides adequate SUPPORT (REASONING and EVIDENCE) for that point; *requires citations*
__ 4. is well ORGANIZED so that the audience can follow the points and examples;
__ 5. employs CLEAR, PRECISE LANGUAGE;
__ 6. is factually ACCURATE and also FAIR, including recognizing objections; *requires citations*
__ 7. is presented in a way that is ENGAGING to the audience;
__ 8. is of a REASONABLE SIZE for the circumstances (not too short or too long);
__ 9. clearly CREDITS OTHERS when their ideas and words are used; *requires citations*
__ 10. Indicates original PREDICTIONS and ultimate findings

Characteristics of a 4.0 Final Manuscript

1. Reads like a scientist wrote it. The author is clearly well-read on the topics they studied, understands their goals and methodologies and communicates that well in a concise, smart, composition.
2. References section contains scientific research papers, recent books and news articles.
3. Figures looks professional with clear and detailed legends.
4. Introduction and Discussion sections have numerous citations and they are used regularly every few sentences. All sections are clearly broken up into sub sections, with sub titles, that focus on each step of the project (such as: Quantitation of DNA Found, DNA purification, PCR analysis).
5. Each step in the research project is addressed individually and thoroughly with context "why and how it was done" as well as evidence "photographs," data sets, mathematical analysis.
6. Error analysis is scientific and thoughtful; citing difficulties found by other researchers in publications, limitations or possibilities that explain problems in data collection.

Characteristics of a 0.0 Final Manuscript

1. Reads like a high school student wrote it. The author is clearly has not read about the topics they studied (even material presented in the course pack), and does NOT understand their project or communicate well in a composition.
2. References section contains anonymous websites and citations are not complete.
3. Figures looks incomplete with short confusing legends.
4. Introduction and Discussion sections have very few citations and all sections are just long paragraphs with no topic sentences and no sub-headings.
5. Each step in the research project is NOT addressed individually and the author seems only focused on quickly being done.
6. Error analysis is trivial where all the human error items listed could easily have been fixed by using controls or doing replications (or even coming to lab more than once a week and paying attention).

(for) Presentations

Group ______________________________

Rubric for scoring Proposal Talk/Movie (LB144)

_____ [P/F/percent] **LITERATURE** RESEARCH: _________________: Student name ________

We used Google Scholar and PubMed to search research papers and reviews on [insert behavior here] and this particular related gene. So far we have found X research papers and Y books that discuss this behavior in ... The first paper is entitled _____ and it demonstrates _______. The second paper is a review ….. We found two research papers where the authors studied genes related to this and found...

_____ [P/F/percent] BACKGROUND ON **BEHAVIOR** ________________: Student name ________

We read papers _______, ________, and _____ and found that [insert communication behavior here] has actions like _____________, and is communication of ___ type, and the behavior is documented with these strategies. We propose to study it in this animal _______ this way...

_____ [P/F/percent] METHODS & capturing behavior ___________: Student name ________

We propose to use smartphone video and boom mic audio to capture this known communication behavior in our _____ animals ______ using this/these method(s) from these published approaches _______, and __________. To further connect our animal research with humans, we propose to study a homologous behavior of _____ and ____ in humans and collect sociological data using these professional published instruments found in this paper _____.

_____ [P/F/percent] METHODS for **genetic** basis : _______________: Student name ________

We read the about this gene which may be connected to this behavior. The full genomic sequence of our gene is available here ________. Here's an image of the DNA sequence. We can also use these published DNA primer sequences from this paper (shown in our slide) to amplify it by PCR using these methods. Once we know the base sequence of the primer we can calculate it's expected annealing temperature using these methods.

_____ [P/F/percent] METHODS use of **CONTROLS**: _________________: Student name ________

As a "experimental control" we will be sure that ___________. We have controls in the behavior studies of animals and humans as well as in the molecular PCR study of our gene. For example, we plan to use positive controls like ….___________ and negative ones for example ___________. The role of the controls are these....

Appendix

Handouts

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Report

Social Transmission of Novel Foraging Behavior in Bats: Frog Calls and Their Referents

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Summary

The fringe-lipped bat, *Trachops cirrhosus*, uses prey-emitted acoustic cues (frog calls) to assess prey palatability [1]. Previous experiments show that wild *T. cirrhosus* brought into the laboratory are flexible in their ability to reverse the associations they form between prey cues and prey quality [2]. Here we asked how this flexibility can be achieved in nature. We quantified the rate at which bats learned to associate the calls of a poisonous toad species with palatable prey by placing bats in three groups: (a) social learning, in which a bat inexperienced with the novel association was allowed to observe an experienced bat; (b) social facilitation, in which two inexperienced bats were presented with the experimental task together; and (c) trial-and-error, in which a single inexperienced bat was presented with the experimental task alone. In the social-learning group, bats rapidly acquired the novel association in an average of 5.3 trials. In the social-facilitation and trial-and-error groups, most bats did not approach the call of the poisonous species after 100 trials. Thus, once acquired, novel associations between prey cue and prey quality could spread rapidly through the bat population by cultural transmission. This is the first case to document predator social learning of an acoustic prey cue.

Results and Discussion

Social learning can expand the foraging repertoire of a given individual and enhance predator foraging success [3–5]. Numerous studies have demonstrated the ability of predators to learn socially about prey cues in the olfactory and visual modalities (see [5] for review). Predator social learning of acoustic prey cues, however, has not been documented. Here we test the ability of the predatory bat, *T. cirrhosus*, to acquire a novel acoustic association for prey via social learning.

T. cirrhosus has a unique ability among bats to prey on frogs by listening to the advertisement calls male frogs produce to attract their mates [1]. In a previous study, we investigated the associations *T. cirrhosus* forms between prey cues (species-specific prey mating call) and prey palatability [2]. Using a fading-conditioning paradigm [6], we were able to rapidly reverse the bats' assessment of palatable and poisonous prey.

Here we ask whether this flexibility is part of the bats' natural foraging repertoire and to what degree novel associations between prey cue and prey quality can be culturally transmitted. To address these questions, we quantified the rate of acquisition of a novel foraging behavior in three learning groups: (a) a social-learning group, (b) a social-facilitation group, and (c) a trial-and-error group. The target foraging behavior was the bats' ability to learn to associate the calls of the sympatric cane toad, *Bufo marinus*, with a palatable food reward. *B. marinus* is both highly poisonous and far too large for a *T. cirrhosus* to eat, so on two accounts it should be an unsuitable prey item. The criterion for task acquisition was flying to and landing on a speaker broadcasting toad calls in three consecutive trials.

We first conducted baseline tests with all bats to determine initial responses to *B. marinus* calls. None of the bats showed any initial response to *B. marinus* calls. We then tested for social learning by allowing an inexperienced bat to observe the foraging behavior of an experienced bat (tutor) that had already acquired the novel association. The first tutor learned to associate toad calls with a palatable food reward via a fading-conditioning technique (for methods, see [2]). Subsequent tutors acquired the association via social learning, such that the test bat in one experiment became the tutor in the next experiment.

The novel foraging association was transmitted successfully among all bats in the social-learning group ($n = 10$). There were two series of sequential interactions, or "chains" (Figure 1). The first chain consisted of two social-learning transmissions among three bats. The third bat died in captivity, and so the fourth bat was conditioned via fading, initiating a second chain of eight social-transmission events among nine bats, at which point we halted the experiment. Bats in the social-learning group acquired the novel foraging task in 5.3 ± 1.7 trials (mean ± SEM, range = 1–11 trials, Figure 2). There was no degradation in the rate of acquisition from bat to bat (Pearson product-moment correlation: $r = 0.314$, $p = 0.377$; Figure 1).

To control for possible motivational effects associated with the mere presence of a second bat [7] in the social-facilitation group, we quantified the rate of acquisition of a test bat housed with an inexperienced conspecific ($n = 5$). In addition, in the trial-and-error group we quantified the rate of acquisition of bats housed alone, when the only possibility for task acquisition was individual learning ($n = 5$). Rates of task acquisition were significantly slower for these two groups in comparison with the social-learning group (Kruskal Wallis test: $H = 15.28$, $p < 0.001$, Figure 2). There was no significant difference in the rate of acquisition between the social-facilitation group and the trial-and-error group (mean trials to acquisition ± SEM: 96.8 ± 3.2 and

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Chain #1

3 5

Chain #2

2 11 1 6 1 9 10 5

Figure 1. Chains of Transfer in the Social Learning Group

Arrows indicate the transmission of the novel foraging behavior; the numbers underneath the arrows indicate the number of trials required to reach acquisition criterion for each bat. After testing, the current test bat became the tutor for the next test bat.

96.2 ± 3.8, respectively. Mann-Whitney U test: $U = 12.0$, $p = 0.881$, Figure 2). If a bat never acquired the novel foraging task, we assigned it an acquisition score of 100 trials; thus, results are conservative measures of task acquisition.

In addition to demonstrating a profound potential for cultural transmission of acoustic-cue associations used in foraging, our results provide evidence as to how such associations might originate in the wild. Most of the bats in the trial-and-error group and the social-facilitation group never learned the novel association between prey cue and prey quality. In each of these groups, one of five bats learned the novel association after more than 80 trials (in the social-facilitation group, this individual reached criterion at 84 trials, and in the trial-and-error group it did so at 81 trials). Thus, although it's rare, bats will inspect calls of poisonous species on their own. This exploratory behavior could enable bats to encounter and track novel sources of prey and could account for the origin of novel associations between prey cue and prey quality in the wild. Such behavior could thus provide a starting point for the rapid cultural transmission that we show here for foraging information among bats.

In other taxa it has been suggested that if mistakes are not costly, it is advantageous for animals to periodically sample their environment. Buchler [8] suggested that wandering shrews (*Sorex vagrans*) make "intentional errors," even when they know where profitable food patches are located, in order to update their knowledge of their surroundings. Likewise, great tits (*Parus major*) have been shown to sample a variety of prey species, even when they have a profitable search image for an abundant prey type [9]. In lowland Neotropical rainforests, there can be extreme fluctuations in the abundance of available prey [10]. In the rainy season, frogs call conspicuously, but in the dry season, frogs are harder to find, and *T. cirrhosus* likely has to rely more heavily on non-anuran prey. It is possible that *T. cirrhosus* uses exploratory behavior in combination with social learning to track local and seasonal changes in prey abundance. Because *T. cirrhosus* uses many sensory modalities to assess its prey (e.g., prey-emitted acoustic cues, echolocation cues, and chemical cues), it is likely that a mistake at one level of prey assessment would be corrected at another level. As such, mistakes should not be costly, and behavioral flexibility should be advantageous.

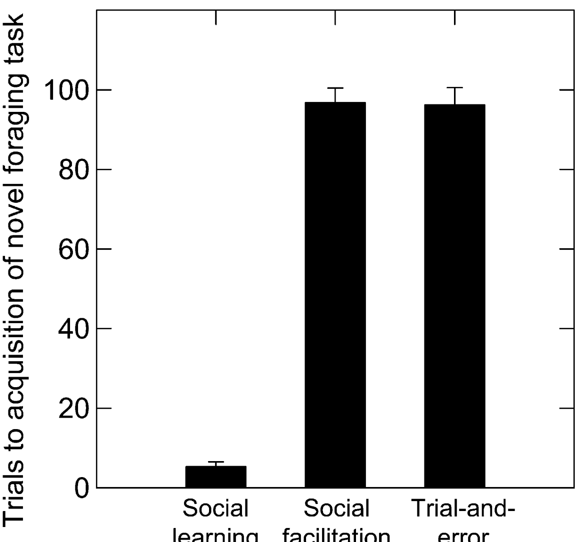

Figure 2. Mean Rates of Acquisition, ± SEM, of the Novel Foraging Task for the Three Learning Groups

Johnston and Fenton [11] found that pallid bats (*Antrozous pallidus*) vary tremendously in their feeding habits, both between and within populations. Indeed, variation seems to be the rule in many species of bat (e.g., [12, 13]). Dietary studies have shown that *T. cirrhosus* preys on a wide variety of prey items, including frogs, insects, fruit, smaller species of bats, and even birds [14–16], with insects composing the largest component of the diet. No seasonal differences in prey consumption were found in analysis of *T. cirrhosus* stomach contents in Brazil, although this could be due to differences in breeding phenology of frogs present in the areas sampled [14]. Further investigation is necessary for determining whether dietary patterns and foraging preferences vary with season and whether this can be linked to learning within social groups.

Our results clearly demonstrate that if one bat forms a novel acoustic association, in this case evaluating a toad call as a signal of palatable prey, then the association can spread rapidly from bat to bat through cultural transmission. All that is required is that bats observe one another feeding in nature.

T. cirrhosus bats are social. They roost in groups of four to 50 or more individuals [17]. Multiple bats can forage simultaneously at a frog chorus [1, 18], and individuals are often captured together in the same mist net at a foraging site (R.A.P. and M.J.R., unpublished data). Thus, in addition to roosting together, *T. cirrhosus* individuals are known to congregate at feeding sites, thus enabling the observation of foraging conspecifics.

Many species of bat have social structures and foraging habits that should facilitate social learning [19]. Bats are long-lived animals, they tend to form stable groups, and they often feed on temporally and spatially fluctuating resources; thus, cultural transmission of foraging information should increase foraging success [19, 20]. Several studies have shown that bats attend to the foraging behaviors of conspecifics. Many species of

insectivorous bats produce loud echolocation calls with distinctive terminal phases as they approach and capture prey. Other individuals eavesdrop on these conspicuous signals and use them to detect aggregations of prey [21, 22]. Likewise, the screech calls of greater spear-nosed bats, *Phyllostomus hastatus*, attract group mates to foraging patches, such as concentrations of flowering balsa [23]. Studies with short-tailed fruit bats, *Carollia perspicillata*, suggest that social learning about temporally fluctuating food resources may even take place at the roost; bats have been shown to use olfactory cues associated with returning roost mates to shape their foraging preferences [20]. These factors suggest that social learning may be common in bats; however, only a handful of studies have demonstrated that bats indeed learn socially about foraging [20, 24–26].

Recent studies have investigated the role of matrilineal kin groups in bat roosting and foraging behavior. Although Kerth et al. [27] found evidence for information transfer about roost sites in Bechstein's bats, *Myotis bechsteinii*, they found no evidence for information transfer about feeding sites. In both the Kerth et al. study and a study of greater horseshoe bats (*Rhinolophus ferrumequinum*), however, radiotracking data demonstrate that mothers and their daughters shared foraging grounds, sometimes for years [27, 28]. Thus, the vertical transfer of foraging-site location from mother to pup could be playing a large role in the foraging dynamics of these bat communities. Although the learning we document in our study is likely entirely opportunistic (the result of one bat eavesdropping on the successful foraging behavior of another), the study of social learning in highly related groups, and especially in mother-pup pairs, should prove an interesting area for further research.

Our study is not designed to distinguish among the mechanisms of social learning [29–32]; however, it is likely that these bats are learning by either stimulus enhancement or observational conditioning. In stimulus enhancement the activity of the tutor draws the observer's attention to the test stimulus [31, 33]—in our experiment, to the toad calls. The observer then forms an association between the stimulus and the reward via individual, trial-and-error learning. Because we altered the speaker location for each trial, we can rule out the possibility that the bats are learning to associate a food reward with a particular spatial location (local enhancement).

In observational conditioning, a type of higher-order conditioning, the observer associates the stimulus with the outcome experienced by the tutor and thus responds more readily to the stimulus itself [31, 34]. In our social-learning treatment, the test bat did not initially attend to the toad calls or to the flight of the tutor bat. In the initial trials, the test bat typically would commence responding with ear motions and head orientation only once the tutor began to consume the food reward, and only in later trials did the test bat respond to the test stimulus. These observations suggest that the sensory cues associated with food consumption by a nearby bat may serve to trigger attention and thus expedite the acquisition of novel foraging associations.

Once the attention of the test bat was elicited, a number of sensory cues could have been involved in the test bat's observation of the tutor. Our experiments were conducted under low-light conditions, such that visual observation was possible. As *T. cirrhosus* approaches a target, the rate of its echolocation calls increases [35]; thus, the test bat could have used the patterns of the tutor's echolocation calls to follow its behavior. It could also have tracked the movements of the tutor bat with its own echolocation calls, and it could have passively listened to the crashing noise produced when the tutor bat landed on the screen. Further investigation is necessary to determine both the learning mechanisms and the sensory cues involved in this social interaction.

The flexibility, exploratory behavior, and social learning we document in this study endow the bats with the potential to respond rapidly to changes in prey conditions. With the catastrophic and worldwide decline of amphibians [36–38], their predators' ability to track such changes becomes increasingly critical. Rather than falling into ecological traps, unable to quickly alter previously adaptive behavior [39], *T. cirrhosus* should be able to respond quickly to changes in the prey community, the extinction of preferred prey items, and the introduction of novel prey species.

Experimental Procedures

Experiments were conducted at the Smithsonian Tropical Research Institute field station on Barro Colorado Island (BCI), Panama, from February to June 2004 and 2005. We captured the bats in mist nets and tested them in a 4.5 m × 4.5 m × 2.5 m outdoor flight cage. We illuminated the flight cage with a 25 watt red light bulb to facilitate our observations of the bats. This light level was within the range of illuminations in which the bats forage. We used a Sony NightShot DCR-TRV340 camera equipped with a Sony HVL-IRH2 infrared light to record all initial and final tests, all social learning trials, and a subset of the social-facilitation and trial-and-error learning trials. Each bat was marked with a passive integrated transponder (PIT tag) and released at its site of capture after testing. All experiments were licensed by the Smithsonian Tropical Research Institute and the University of Texas at Austin (IACUC #04113002).

Stimulus Presentation

We broadcast calls of *B. marinus* from a Dell Inspiron 8100 computer, a SA-150 Realistic amplifier, and 40-1040 Radio Shack speakers. To approximate the natural call intensity of *B. marinus* in the wild, we broadcast the calls at an amplitude of 75 dB SPL (re. 20 μP) measured at a distance of 1 m from the speaker. Most of the energy in *B. marinus* calls falls between 548 and 708 Hz; the frequency response of these speakers is flat for these frequencies. To ensure that the bats responded to the acoustic stimulus broadcast and not to the speaker itself, we concealed one to five speakers beneath a 1.5 m × 1.5 m screen covered with leaf litter and randomly repositioned the speakers between trials. To ensure that the bats were responding to the toad calls per se and not to other noises associated with the speaker, in a subset of the trials we turned on one of the control speakers and broadcast a sound file of silence. The bats never approached control speakers. Toad calls were broadcast for 60 s or until the test bat landed on the speaker, whichever came first. Trials were conducted in approximately 10 to 15 min intervals with a maximum of 20 trials per night.

Food Rewards and Motivation Levels

Because frogs are protected on BCI, small bait fish were purchased, frozen, thawed, and used as food rewards for the learning trials. The bats readily consumed the fish. To ensure that the bats were not responding to extraneous cues associated with the rewards, in all learning trials we placed multiple rewards in random locations on the screen. Bats only approached food rewards placed on active speakers.

T. cirrhosus picks up its prey in its mouth and flies with it to a nearby perch to consume it. *T. cirrhosus* generally carries only one prey item at a time. We placed many pieces of fish on the active speaker so that if both the tutor and the test bat landed on the speaker in a given trial, each bat would receive a food reward. We closely monitored the bats' food intake to ensure high motivation levels, and trials were conducted only when bats were motivated to feed.

Between trials, we periodically broadcast a probe stimulus known to elicit response in *T. cirrhosus* (the calls of a preferred prey species: either a complex túngara frog call or a chorus of túngara frog calls [1, 40]). If the test bat did not respond to the probe stimulus, we could infer that a lack of response to the toad calls could have been due to factors other than the salience of the test stimuli (i.e., handling stress, fear of new environment, satiation). Bats always responded readily to the probe stimuli in all probe tests.

Summary of Trials

1. Initial Trials

To determine its baseline response to toad calls, we first tested each bat alone, without rewards on the speaker. Toad calls were broadcast for 60 s or until the bat approached and landed on the speaker, whichever came first. Three initial tests were conducted for each bat.

2. Learning Trials

Three types of learning trials were conducted: (a) social learning, (b) social facilitation, and (c) trial and error. Learning trials were conducted until criterion (flying to and landing on the speaker in three consecutive trials) had been reached or until 100 trials had been conducted, whichever came first. Food rewards were placed on the speaker in all learning trials.

3. Final Trials

After learning trials, we removed the tutor bat and conducted three final trials with the test bat alone. The protocol for final tests was identical to initial tests. Final test results mirrored criterion results: all bats that reached criterion in the learning trials responded to toad calls by flying to and landing on the speaker in all three final trials. No bat that failed to reach criterion in the learning trials responded in the final trials.

Supplemental Data

One supplemental figure is available with this article online at http://www.current-biology.com/cgi/content/full/16/12/1201/DC1/.

Acknowledgments

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RECOGNITION AND SELECTION OF SETTLEMENT SUBSTRATA DETERMINE POST-SETTLEMENT SURVIVAL IN CORALS

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Abstract. Habitat recognition and selective settlement by dispersive propagules greatly increases the post-settlement survival chances of sessile organisms. To better understand the key role some species can play in the structure of highly complex coral reef ecosystems, we compare the role of two independent, but sequential, processes: settlement choice and post-settlement survival. This study describes the chemical and physical recognition and ranking of specific settlement substrata by coral larvae. Several species of crustose coralline algae (CCA) are known to induce coral settlement; however they also employ physical and biological anti-settlement defense strategies that vary greatly in effectiveness. We examine the interactions between settling larvae of two common reef building coral species (*Acropora tenuis* and *A. millepora*) and five species of CCA (*Neogoniolithon fosliei*, *Porolithon onkodes*, *Hydrolithon reinboldii*, *Titanoderma prototypum*, and *Lithoporella melobesioides*) that co-occur on reef crests and slopes of the Great Barrier Reef, Australia. Distinct settlement patterns were observed when coral larvae were provided with a choice of settlement substrata. Settlement on the most preferred substratum, the CCA species *T. prototypum*, was 15 times higher than on *N. fosliei*, the least preferred substratum. The rates of post-settlement survival of the corals also varied between CCA species in response to their anti-settlement strategies (shedding of surface cell layers, overgrowth, and potential chemical deterrents). Rates of larval settlement, post-settlement survival, and the sensitivity of larvae to chemical extracts of CCA were all positively correlated across the five species of CCA. Nonliving settlement substrata on coral reefs is sparse; consequently the fact that only a few CCA species (notably *T. prototypum*) facilitate coral recruitment, has important implications for structuring the reef ecosystem.

Key words: coral; coralline algae; defense; Great Barrier Reef, Australia; metamorphosis; recruitment; settlement; shedding; substrata; survival.

Introduction

The distribution and abundance of sessile organisms is often governed by the settlement of their mobile propagules. For marine organisms, larval settlement and early post-settlement survival can explain much of the variance in adult populations (Raimondi 1990, Palma et al. 1999). Settlement strategies of mobile larvae vary from passive, indiscriminate settlement to active substratum selection, which can help minimize the chances of settling in unsuitable habitats (e.g., Grosberg 1981). One of the recurrent themes of marine ecology has been the tendency to underestimate the capacity of larvae to influence their fate (Young and Chai 1985); however recent research suggests habitat selection by larvae at settlement has a strong influence on post-settlement survival and adult distribution patterns (Mundy and Babcock 1998, Baird et al. 2003).

Coral reefs are highly diverse marine communities constructed and dominated by sessile organisms that disperse via a planktonic larval stage in their early life history. Microhabitats on coral reefs conductive to coral larval settlement and persistence are limited. Unoccupied primary substrata are rare and most other surfaces such as live corals, filamentous turf algae, and sediment are poor habitats for settlement and survival. Coral planula choose their site of permanent attachment based upon physical factors such as light, salinity, water motion, depth, surface orientation, and sedimentation (Maida et al. 1994, Mundy and Babcock 1998, Raimondi and Morse 2000). Living surfaces of nongeniculate or ''crustose coralline'' algae (CCA; Rhodophyta, Corallinaceae) can aid in the survival of settlers by excluding other space competitors and providing protection from sediment entrapped in turf algae (Babcock and Mundy 1996, Ruiz-Zarate et al. 2000). The suitability of a substratum as attachment site is however primarily determined by chemical and/or biological surface properties, such as surface films of algae, diatoms, or bacteria (Morse et al. 1988, Johnson et al. 1997, Raimondi and Morse 2000, Baird and Morse 2004).

Settlement and metamorphosis in many scleractinian corals is induced by external biochemicals (morphogens) associated with living CCA (Morse et al. 1988,

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PLATE 1. Example of an anti-settlement defense mechanism in crustose coralline algae (CCA): epithallial shedding of >80% of thallus surface, removing most of the two-day-old coral settlers on the CCA species *Neogoniolithon fosliei* in a single sloughing event. Two of the settlers are marked by arrows. Scale: ~1:1. Photo credit: L. Harrington.

1996, Heyward and Negri 1999). These morphogens were identified as cell wall bound polysaccharides (Morse and Morse 1991). Although many species of CCA are capable of inducing metamorphosis in laboratory experiments, each species has specific characteristics that could influence its ecological relevance to habitat selection by the larvae. Larvae of the brooding Caribbean coral *Agaricia humilis* exhibited species-specific settlement preferences among five unidentified CCA species in laboratory experiments (Morse et al. 1988). Later research showed that larval settlement in this species was associated with the common CCA *Hydrolithon boergensii* (Raimondi and Morse 2000). A recent field study on the Australian Great Barrier Reef (GBR), has demonstrated a species-specific preference of broadcast-spawning corals to recruit on the coralline alga *Titanoderma prototypum* (R. Steneck, E. Turak, L. Harrington, and T. Done *unpublished manuscript*). The field observation of a high abundance of coral recruits associated with *T. prototypum* suggests this species may play a critical role in facilitating coral recruitment on the GBR.

Coral larvae may be induced to settle by properties of the substrata; however, subsequent mortality of recruits, caused by active substratum defenses, may result in the high rates of juvenile mortality. The potential for CCA to play a negative role in coral recruitment by increasing post-settlement mortality and reducing growth rates of coral recruits has not been considered. Many species of CCA eliminate newly settled organisms by shedding epithallial cells, i.e., sloughing (e.g., Keats et al. 1997). Overgrowth is also an important strategy of interference in the competition between space-limited organisms (Sebens 1986, Airoldi 2000). Thick CCA can easily overgrow newly settled corals (Maida et al. 1994, Dunstan and Johnson 1998), and even established adult corals (Antonius 2001). Chemical defense, in particular allelopathy, is a third widespread antifouling strategy used by many sessile organisms (Maida et al. 1995, Suzuki et al. 1998). Although chemical cues are known to induce settlement and metamorphosis of coral larvae on CCA, the potentially allelopathic nature of some of their metabolites have so far received little attention.

To better understand the key function some CCA species can play in the structure of coral reef ecosystems, we have examined the role and relationship of two independent but sequential processes: selective settlement and post-settlement survival of coral larvae. We quantified the settlement preferences of larvae of the two reef building corals *Acropora millepora* and *A. tenuis* on horizontal and vertical surfaces of living and dead CCA species, and on two inert substrata. We then quantified survivorship of newly settled corals on these substrata to determine if species-specific habitat selection influences post-settlement mortality levels. We also examined the chemically mediated induction of coral settlement by different species of CCA, and assessed the relationship of this process with that of selective settlement and post-settlement survival.

METHODS

We examined how both physical and chemical attributes of CCA influence larval settlement and metamorphosis (Experiment 1a and b) and post-settlement survival of juvenile corals (Experiment 2) in a controlled laboratory environment. We also examined the presence of chemical inducers for metamorphosis (morphogens) in each species of CCA (Experiment 3). Experiments were conducted with two species of the coral genus *Acropora*, representing the dominant genus on the Great Barrier Reef (GBR) collected from three spawning events, and five species of common CCA found on offshore reefs of the GBR. Settlement experiments were performed in outdoor aquaria at the Australian Institute of Marine Science (AIMS, Townsville, Queensland), and at the Lizard Island Research Station (LIRS, Great Barrier Reef, Queensland).

Obtaining coral larvae

Experiments 1a and 2.—Ten mature colonies of *Acropora tenuis* were collected prior to spawning from

Nelly Bay, Magnetic Island (19°10′ S, 46°51′ E). Spawning occurred on 2 November 2001.

Experiment 1b.—Six mature colonies of *Acropora millepora* were collected from Lizard Island (14°40′ S, 145°26′ E). Spawning occurred on 21 November 2002.

Experiment 3.—Six mature colonies of *A. millepora* (Ehrenberg 1834) were obtained from the Whitsunday Islands (20°15′ S, 148°50′ E), which spawned at AIMS on 5 December 2001.

For all experiments the gametes were fertilized and larvae cultured as per Heyward and Negri (1999).

Collection of non-geniculate crustose coralline algae

Experiments 1a, 2, and 3.—CCA were collected from the slopes of Davies Reef (18°50′ S, 147°742′ E) at 3–7 m depth. Five common species of CCA were collected on scuba using hammer and chisel: *Hydrolithon reinboldii*, *Neogoniolithon fosliei* (see Plate 1), *Porolithon onkodes*, *Lithoporella melobesioides*, and *Titanoderma prototypum*. The identity of each specimen was verified under a dissecting microscope using reproductive and vegetative morphological and anatomical features as diagnostic characters (Gordon et al. 1976, Adey et al. 1982). The epithallial surface area of each fragment was determined using the foil wrap technique (Marsh 1970; median area: 16 ± 0.9 cm^2, mean ± 1 SE). CCA fragments were maintained at AIMS in an outdoor 1000-L flow-through tank under a pivoted PVC trough that tipped ~25 L of seawater every ~2 min to mimic a high-energy reef environment. One week after collection, the fragments were cleaned to remove all epibionts prior to experimental manipulation.

Experiment 1b.—The same CCA species (except for *L. melobesioides*) were collected at Lizard Island from the reef crest and slope between 3 and 7 m depths, and maintained in outdoor 25-L flow-through tanks at LIRS. Total thallus surface area averaged 11 ± 0.4 cm^2.

Substratum-specific settlement rates

Experiment 1a: Acropora tenuis *larvae.*—This experiment was performed to determine the effects of substratum type, the health of the CCA, and substratum orientation on settlement rates of *A. tenuis* larvae. Half of the CCA fragments of each species were kept alive, while the other half were killed by rinsing in fresh water for one hour and sun drying for five hours. Two additional treatments of inert substrata were prepared by cutting similarly sized fragments from unglazed five-day conditioned terracotta and from dead skeleton of the massive scleractinian coral *Porites* sp. One-half of the live and dead fragments were embedded onto glass slides using underwater epoxy putty (AquaStik, Aquasonic, Ingelburn, New South Wales, Australia) allowing for vertical deployment. One replicate of each treatment (both live and dead vertical and horizontal fragments of five CCA species and two inert substrata) was placed in each of five aerated aquaria containing unfiltered seawater. These aquaria were placed within a large outdoor flow-through tank, acting as a water bath, under 90% shading to minimize overgrowth by filamentous algae. Nine days after fertilization, when the majority of larvae in the culture were competent to settle, ~8000 larvae were added to each of the five aquaria, where they were simultaneously presented with all substrata treatments. Three days after larval transfer, when many of the larvae had either settled or ceased swimming, the numbers of metamorphosed corals were determined on each fragment, using the criteria of Heyward and Negri (1999).

Experiment 1b: Acropora millepora *larvae at LIRS.*—To create stable horizontal settlement surfaces, all CCA fragments were embedded into plastic petri dishes with nontoxic underwater epoxy (Z-spar Splash Zone Compound, Kop-Coat, Pittsburgh, Pennsylvania, USA). Sixteen replicates of each of the CCA species, and fragments of terracotta tiles of similar sizes, were placed into eight replicate aquaria containing unfiltered seawater. At five days of age, ~3000 larvae were added to each of the aquaria.

Post-settlement survival

Experiment 2: Survival of Acropora tenuis *spat in the laboratory.*—In order to assess early post-settlement survivorship, the number of *A. tenuis* spat needed to be increased on all substrata except for *T. prototypum*. To achieve this, all *T. prototypum* fragments were removed from the aquaria after three days of exposure to larvae. Within three more days, the number of settled and metamorphosed spat on all remaining substrata had increased by 19% (an additional 400 spat). After a total of six days exposure to larvae, all CCA fragments were transferred from the aquaria to perforated plastic trays and placed within the outdoor flow-through 1000-L tank with unfiltered seawater and the water-dumping PVC trough to remove sloughed cell layers. Survivorship of *A. tenuis* on the fragments was examined under a dissecting microscope daily for the first 30 days, and once more 6 months later.

Investigating chemical responses

Experiment 3: Larval metamorphosis in response to CCA morphogen concentrations.—We examined whether each CCA species contained extractable chemical inducers to trigger metamorphosis (morphogens) in the coral larvae tested. Sterile extracts from each of the CCA species, terracotta, and *Porites* sp. were prepared by individually grinding a known wet mass of chips of each substratum in methanol (HPLC grade). The slurry was passed through 0.45-μm, methanol washed nylon filters (47 mm diameter, Gelman Science, Ann Arbor, Michigan, USA) and the filtrate retained. The extraction process was repeated and the filtrates combined and evaporated to dryness under N_2. The extracted residues were then resuspended in 0.2-μm filtered seawater to a stock concentration equivalent to

500 mg CCA/mL and stored frozen. Control extracts were prepared as described but with addition of either ground *Porites* or terracotta, or without the addition of ground substrata.

To test for settlement competency, larvae were exposed each day to 5 × 5 mm chips of *P. onkodes* (Heyward and Negri 1999). High levels of metamorphosis (>70%) were achieved after six days in 2001 and four days in 2002; hence the CCA extract induction experiments were performed with seven- and five-day-old larvae, respectively.

Larval metamorphosis assays were performed in sterile 10-mL wells (six-well culture plates, Nunc, Hongo, Bankyo-ku, Tokyo, Japan) maintained in a constant temperature room at 120 μmol quanta·m^{-2}·s^{-1} for 12 h/d, and strong fan-forced air flow to maximize gas exchange on the seawater surface in the wells. The temperature was set to 28–29°C in 2001 and 26–27°C in 2002. Coral larvae (n = 10–20) were introduced to each well containing 0.2-μm filtered seawater and the CCA extract to a final volume of 10 mL. The concentration range of CCA extract added to the wells was 0–50 mg CCA/mL seawater, with the concentration based upon the original wet mass of CCA extracted to enable comparison between species. For the highest extract concentration (50 mg/mL), the mass of organics added was ~150 μg total extract/mL. Six replicate wells were used for each treatment. Early-stage settlement and metamorphosis was assessed after 36 h using a dissecting microscope.

Statistical analyses

Experiment 1a and b.—Log-linear models were used to analyze larval settlement rate as a function of settlement substratum (five CCA species and two controls), status of CCA fragment (live and dead), orientation (horizontal and vertical), and sampling year. The initial model involved main effects and all interactions. The final model was selected by backward elimination of nonsignificant terms ($P > 0.05$). Overdispersion was present in the data (McCullagh and Nelder 1989), and F ratio tests based on the mean square deviance were used to assess the significance of effects.

Experiment 2.—For the survival data, we investigated how the instantaneous probability of larval survival at a given point in time (the hazard) varied with the treatments. The use of parametric and Cox proportional hazards models were assessed; however the model assumptions could not be satisfied for either approach. Since all censoring (discontinuation of observations on surviving larvae) occurred at the end of the experiment, it was possible to analyze the hazard for each period without the censoring resulting in biased estimates. The response variable was the number of deaths during a period divided by the number alive at the beginning of the period. Log-linear models were used with the hazard as the response, and the explanatory variables were settlement substratum, orientation, day, and aquaria nested in species. The variation due to aquaria was used as the error term for effects not involving day, and the mean deviance was used to assess all other effects (McCullagh and Nelder 1989). Partial effects plots were used to show the results; these plots show the effect(s) of one variable in a model adjusted for the effects of all others.

Experiment 3.—To investigate chemical induction of metamorphosis, the proportions of metamorphosed coral larvae were modeled using generalized additive models (Hastie and Tibshirani 1990) for the five species of CCA. Since the responses were proportions that varied smoothly with dose, we used binomial models with smoothing splines. The dose level at which the maximum response was observed was estimated from the fitted response curves. Confidence intervals for the maximum response dose for each species were obtained by taking bootstrap samples of the data ($N = 1000$), refitting the models, calculating the maximum response dose for each bootstrap sample, and taking the median as an estimate of maximum responses and 10th and 90th percentiles of the distributions as the end points of 90% confidence intervals (Davison and Hinkley 1997). Differences between the species were assessed using permutation tests ($N = 1000$).

Synthesis.—The properties of the five species of CCA used in the three experiments were related to each other by examining correlations between the species effects across the three pairs of experiments.

RESULTS

Experiment 1a and b: Substratum-specific settlement rates of Acropora tenuis *and* A. millepora *larvae.*—Nine-day-old *Acropora tenuis* and five-day-old *A. millepora* larvae were observed to settle on all substrata tested, regardless of species, orientation, and health status, but the number of settling larvae varied greatly between the different treatments (Fig. 1). Log-linear analyses of the coral settlement rates onto CCA substrata revealed all interactions to be nonsignificant ($P > 0.05$). Differences in rates of settlement between the two sampling years were also nonsignificant, while all other main effects were significant: species ($F_{7,77} = 7.12$, $P < 0.001$), orientation ($F_{1,77} = 13.5$, $P < 0.001$), and status ($F_{1,77} = 41.0$, $P < 0.001$). Settlement varied greatly across the five species (Fig. 1), being 14.9 times higher (95% CI = (5.5, 40.5)) on *T. prototypum* (the best settlement inducer) than *N. fosliei* (the weakest settlement inducer). In all CCA species, living fragments induced greater settlement and metamorphosis than dead fragments, settlement being 9.4 (3.7, 23.6) times higher for live rather than dead substrata. On all substrata, settlement on horizontal surfaces was 2.8 (1.5, 5.1) times higher than on vertical surfaces.

Experiment 2: Survival of Acropora tenuis *spat.*—Survival experiments revealed a very similar pattern to that observed for settlement. The variation in survival of coral spat between substrata was extreme (Fig.

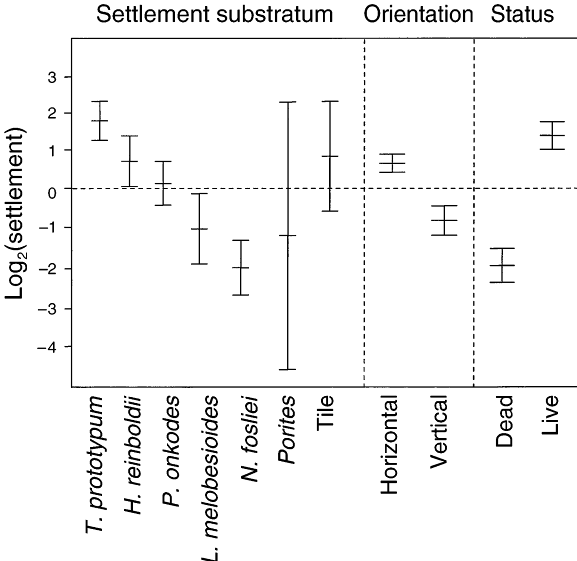

FIG. 1. Partial effects plots of settlement densities of coral larvae, showing the effects of various substrata (live and dead crustose coralline algae species (CCA) and inorganic substrata) at horizontal and vertical orientation (Experiment 1). The effects are plotted on $\log_2$ scale, and thus an increase or decrease by one unit corresponds to a doubling or halving in settlement numbers, respectively (e.g., settlement on live CCA is $2^{3.2} = 9.2$ times as high as on dead CCA). Error bars represent ±1 SE.

2). Mortality was 100% on *N. fosliei* after three days, and <50% on two other substrata (*T. prototypum* and inert tile) after 22 days (Fig. 2). The log-linear analysis showed strong differences between substrata ($F_{7,584} = 23.04$, $P < 0.001$) and moderate differences due to days ($F_{(2,584)} = 5.50$, $P = 0.007$), orientation ($F_{(1,584)} = 5.48$, $P = 0.020$), and the interaction between substrata and days ($F_{(13,571)} = 4.47$, $P < 0.001$). This interaction was evident in the crossing of survival curves (Fig. 2), but was not particularly strong; thus averaged effects for substratum and days were obtained by dropping the interaction from the model. The probability of mortality (hazard) varied greatly across the five species, and was 36.5 (20.1, 66.1) times higher for *N. fosliei* than for *T. prototypum*: the latter did not differ from tile (Fig. 3). For orientation, vertical tiles exhibited higher relative hazards (1.49, (1.13, 1.97)), and the relative hazard increased over the first 10 days but plateaued thereafter. After 240 days, survival of coral recruits on tiles and on *T. prototypum* was 20.1% (6.4, 42.0) and 24.2% (8.2, 52.1), whereas no survivors were observed on any of the other CCA species.

Three types of spat mortality were observed: (1) shedding of CCA thallus layers, thereby removal of the spat (see Plate 1), (2) overgrowth of the spat by CCA, and (3) mortality agents independent of visible/obvious CCA influences as estimated by mortality on the terracotta tiles (i.e., skeleton present, spat itself dead). Sloughing was seen in three CCA species: *N. fosliei*, *P. onkodes*, and *H. reinboldii*. In all colonies of *N. fosliei*, the sloughing of large sheets of epithallial cells removed >50% of the thallus in single events (see Plate 1; also see Appendix: panel A). In contrast, small flakes or individual cells of thallus filaments were continuously shed in *P. onkodes* and *H. reinboldii*, resulting in continuous loss of spat over the course of the first five days (Fig. 2). After 22 days, the overall post-settlement mortality was greater in *P. onkodes* than in *H. reinboldii*, as some proportions of the surfaces appeared to remain unshed over extended periods of time

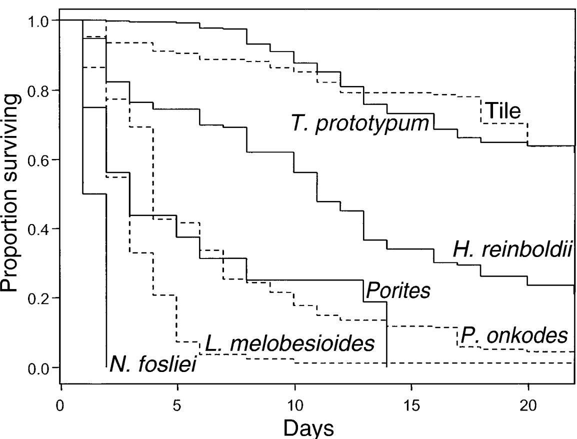

FIG. 2. Survival plots of coral settlers over 22 days (Experiment 2). The individual profiles correspond to proportions of surviving settlers on the five crustose coralline algae species and the two control substrata (*Porites* and tile) over time.

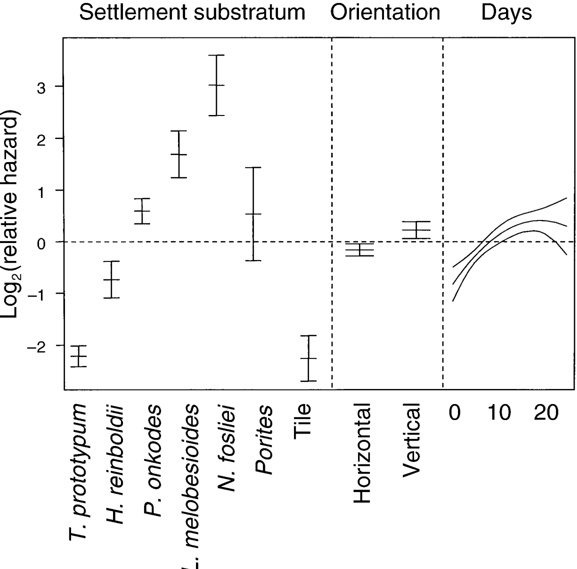

FIG. 3. Partial effects plots for relative hazard of coral spat (Experiment 2). The effects are for the substrata (crustose coralline algae species and inorganic substrata), orientation (horizontal and vertical), and the number of days since the start of the experiment. The effects are plotted on $\log_2$ scale; for interpretation see Fig. 1. Error bars represent ±1 SE.

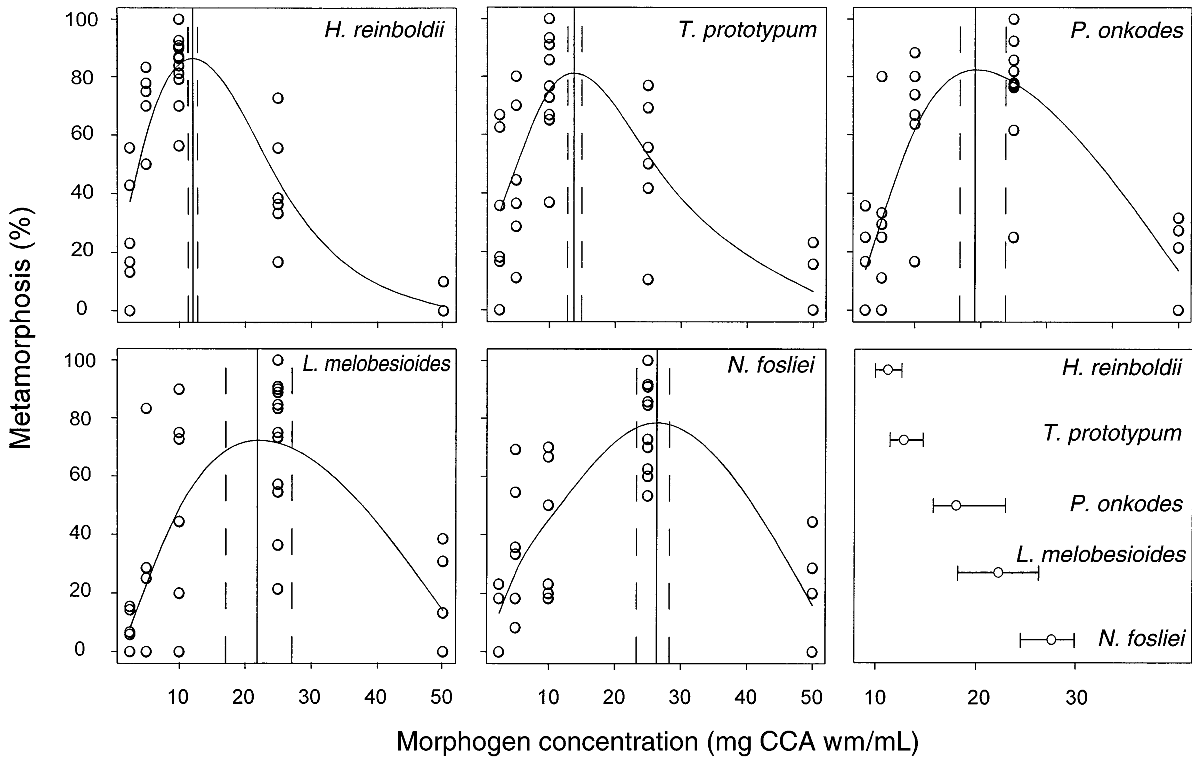

FIG. 4. Settlement and metamorphosis of coral larvae in response to concentrations of methanol-soluble extracts of crustose coralline algae (extract concentration based on mg CCA wet mass [wm]/mL seawater; Experiment 3). Smoothing splines were fitted to the response data, solid vertical lines indicate curve maxima (determined by bootstrapping analyses), and dashed lines are 10% and 90% confidence percentiles. Nonoverlapping bars in the final summary plot indicate significant differences ($P < 0.01$) between species in the locations of the maxima.

in the latter species. Although *L. melobesioides* was not observed shedding, the initial drop in density during the first three days suggests that the spat may have been lost in an unrecognized sloughing event. *T. prototypum* was also not observed to shed.

Overgrowth was another observed cause of mortality in coral spat. *T. prototypum* and *P. onkodes* were able to completely overgrow spat aged 7–30 days (0.5–2 mm in diameter), employing two distinctly different strategies. In *P. onkodes*, the growing margin grew up and over the spat (Appendix, panel C). In *T. prototypum*, the growing margins approached from all directions in very thin layers, surrounding the spat and reaching around/above the basal plate of the coral until they had completely overgrown the entire coral (Appendix: panel B).

Experiment 3: Larval metamorphosis in response to CCA morphogen concentrations.—Extracts of each of the five species of CCA induced settlement and metamorphosis in *A. millepora* larvae, confirming that methanol-soluble morphogens were present in all CCA species tested. The maximum level of metamorphosis occurred at different concentrations for the five species (Fig. 4, permutation test, $P < 0.001$), with levels of induction peaking at extract concentrations equivalent to 10–25 mg CCA/mL (Fig. 4). The maxima for *H. reinboldii* and *T. prototypum* induction occurred at lower extract concentrations (10 mg CCA/mL) than the remaining three species (25 mg CCA/mL), whereas there were no differences within each of these two groups. At low to medium concentrations, larvae were actively swimming and appeared healthy, whereas at the highest concentration used (50 mg CCA/mL), some of the larvae appeared unusually elongated and immobile. Control extracts (from terracotta, *Porites*, or no substratum) induced no metamorphosis over the concentration range examined.

Synthesis of the three experiments

Pairwise plots of the estimated effects for the five species showed strong positive relationships between CCA extract-induced metamorphosis, settlement, and survival (Fig. 5). High levels of coral settlement were related to high levels of survival (low relative hazards, $r = 0.99$, $P < 0.001$) and to high sensitivity to the corresponding CCA chemical inducer ($r = 0.91$, $P < 0.030$). Survival was highest on CCA species that induced highest levels of metamorphosis at low concentration of CCA extracts ($r = 0.92$, $P < 0.026$). The least chosen settlement substrata required higher concentrations of the species-specific CCA extract and exhibited low post-settlement survival, indicating that

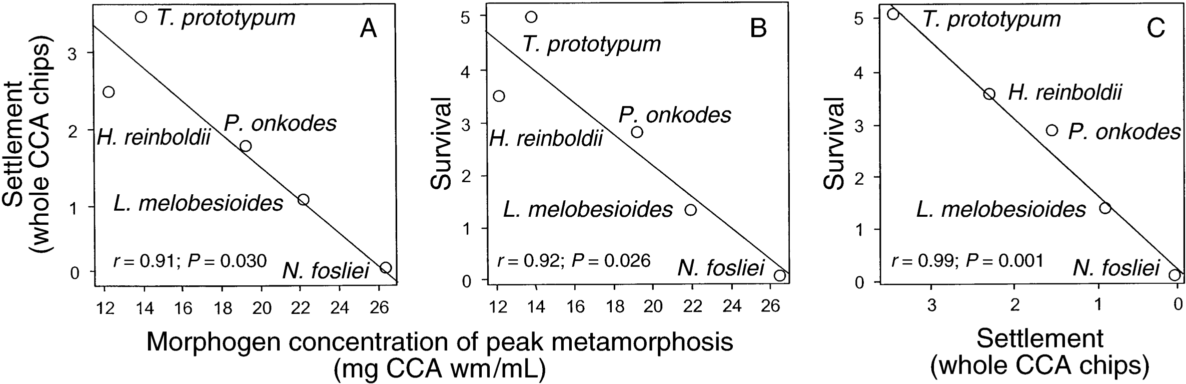

FIG. 5. Relationships between relative effects of sensitivity to chemical induction extract concentrations (Experiment 3), settlement (Experiment 1), and survival (Experiment 2) of coral larvae for the five species of crustose coralline algae (CCA). (A) Rate of settlement on whole live CCA fragments vs. sensitivity to CCA extract concentration. (B) Survival vs. sensitivity to CCA extract concentration. (C) Survival vs. settlement on whole, live CCA fragments. Sensitivity to chemical induction of metamorphosis was estimated as the concentration (%) at which peak induction occurred (Fig. 4), settlement effects were estimated as the relative probability of settlement ($\log_2$) (Fig. 1), and survival effects were estimated as the inverse of the relative hazard ($\log_2$).

early presettlement inducers and behaviors allow larvae to select and settle on substrata, thus enhancing their future survival probabilities.

Discussion

CCA have previously been identified as preferred recruitment substrata for scleractinian corals (Morse et al. 1988, Raimondi and Morse 2000). Our research confirms this role for certain key CCA species of the Great Barrier Reef but not for other, more abundant, CCA species. We demonstrated that coral larvae are able to recognize and choose CCA species that have the least effective antifouling defenses. Active selection of permanent attachment onto certain substrata leads to an increase in survival over the first four weeks following settlement. The highest rates of settlement on *T. prototypum* coincided with lowest post-settlement mortality, and settlement was triggered by one of the lowest CCA extract concentrations. In contrast settlement induction and survival was lowest on the most abundant CCA species in the GBR, *N. fosliei* and *P. onkodes*. Therefore, a few select species of CCA can greatly contribute to controlling the fine-scale distribution patterns of corals within a reef ecosystem.

Settlement cues for coral larvae

Settlement rates in *A. tenuis* and *A. millepora* clearly differed depending on: (1) CCA species, (2) orientation of the substrata, and (3) whether the CCA was alive or not. Strong chemical inducers for settlement and metamorphosis (morphogens), such as the cell wall-bound polysaccharide identified from *H. borgesenii* (Morse and Morse 1991) should facilitate the selection of settlement sites that enhance post-settlement survival. Previous studies investigating the source and potency of coral morphogens have been performed using substrata or CCA extracts in isolation (Morse et al. 1988, 1996, Morse and Morse 1991, Heyward and Negri 1999). Those experiments demonstrate that extracts of most species of CCA tested contain morphogen(s) able to induce high levels of settlement and metamorphosis of coral larvae within 24 hours. Our results also show that, each of the CCA species tested contained a strong methanol-soluble morphogen, and that the potency of the CCA extract differed between species. The comparison of extract potencies may not reflect field behavior due to: potential differences between species in the solubility of active compounds, their surface area–mass ratios, and possible inhibition by co-extracted metabolites. When provided a choice of settlement substrata, larvae preferentially settled upon *T. prototypum*, the species that, along with *H. reinboldii*, exhibited the most potent CCA extract. Strong correlations between settlement preference on CCA fragments and extract potency were observed for all CCA species tested. These results suggest that coral larvae may be able to recognize subtle differences in chemical signatures or respond to different concentrations of morphogen on the surface of the CCA and use these signals when selecting their attachment site. Further research into the identity and specific concentrations of morphogen(s) associated with each of the CCA species is needed to confirm this.

Physical factors also contributed to determining the choice of attachment site. Settlement by *A. tenuis* larvae was significantly higher on horizontal opposed to vertical surfaces. This confirmed previous observations that larvae prefer upper surfaces for settlement, so long as these surfaces are free of sediment, algal growth, and grazing (Mundy and Babcock 1998). Physical conditions such as light and sedimentation are known to strongly influence settlement orientation in corals. For example, preferences shift from under surfaces in shallow water or at high light levels, to vertical surfaces

and then upper surfaces in deep water (Babcock and Mundy 1996, Baird and Hughes 2000). Again, this settlement behavior has selective advantages, despite the slower growth rate in a shaded position, as it reduces post-settlement mortality by grazing, algal growth, and sedimentation (Raimondi and Morse 2000).

The clear preference for larvae to settle on live CCA rather than dead CCA of the same species, indicates that chemical or biological properties of living CCA surfaces are more effective in inducing coral settlement, or that compounds released upon death inhibit settlement to some degree. This is supported by previous laboratory assays where larvae were induced to metamorphose by dead CCA but attached to the polystyrene walls of the test wells, instead of the dead CCA chips (Heyward and Negri 1999). Clean terracotta tiles do not contain chemical morphogens and need to be ''conditioned'' in seawater to enable the establishment of a microbial/algal biofilm before settlement takes place. The terracotta tiles used in these experiments were conditioned in unfiltered seawater for five days, and presented only a very immature biofilm; nevertheless they were a highly preferred settlement substratum, possibly due to early biofilm development on the tile surface (Webster et al. 2004). Alternatively, the larvae may have encountered water-borne CCA morphogens, or morphogens on nearby CCA (possibly through an amplifier pathway similar to that described in the larvae of the abalone *Halotis rufescens*; Morse and Morse 1984), and then moved along to metamorphose on the inert surface of the tile. Living CCA surfaces can aid in the survival of settlers, by excluding other space competitors, and, by providing protection from turf algae and the sediment entrapped in turf algae (Babcock and Mundy 1996, Ruiz-Zarate et al. 2000). However, while CCA can reduce the mortality of newly settled corals, these recruits also need to survive the defense strategies of the CCA, which include chemical deterrence, overgrowth, and sloughing.

Anti-settlement defense strategies of CCA

Allelopathic compounds are employed by certain CCA species as natural antifoulants (Masaki et al.1981, Suzuki et al. 1998, Degnan and Johnson 1999). In the present study, extracts from each CCA species induced maximum settlement levels of 80% at low to medium extract concentrations (Fig. 4); however, higher concentrations (50 mg CCA/mL) inhibited settlement and metamorphosis. Larval elongation and reduced searching activity at the high extract concentrations indicated the presence of either nonspecific settlement inhibitors co-extracted by methanol, or dissolved allelopathic compounds. As coral larvae successfully settled on the live fragments of each of the CCA species tested, it is unlikely that these species contain or release allelopaths against coral settlement in environmentally effective concentrations.

Overgrowth has been described in several CCA species as a strategy to outcompete fouling organisms including newly settled corals (Babcock and Mundy 1996, Dunstan and Johnston 1998; see *Results*), and adult corals (Antonius 2001). However, our data indicate that overgrowth by CCA plays a relatively minor role in the survival of coral settlers, as the number of overgrowth events was small. For example, after 6 mo the density of coral spat on *T. prototypum* was 24% of the initial level, despite observed overgrowth of recruits by this CCA, and not significantly different from that on inorganic terracotta tiles (20%).

Sloughing is a process by which some species of CCA shed off their upper epithallial layers thereby removing fouling organisms (Masaki et al. 1984, Keats et al. 1997). Sloughing of individual epithallial cells was the most effective antifouling strategy used by *N. fosliei*, *P. onkodes*, and *H. reinboldii*. Observations of incipient fouling in many species of living CCA, followed by the synchronous sloughing of whole epithallial layers (*Sporolithon* spp. and *Neogoniolithon* spp.) or a sloughing of individual epithallial cells in a spatially sequential fashion (*Neogoniolithon mammillare*) or diffuse fashion (*Porolithon* and *Hydrolithon*), indicate the importance of this process in CCA (Masaki et al. 1984, Johnson and Mann 1986). Similar antifouling strategies are widespread in marine and terrestrial plants; for example, seagrasses and laminarian kelps erode at the older apical regions to remove epibionts (Sand-Jensen and Borum 1991, Littler and Littler 1999). Scanning electron microscopy revealed that the epithalli of *T. prototypum* are usually intact, without peeling flakes. This anatomy and mode of growth appears to be a key to the high post-settlement survival on *T. prototypum*.

We are unable to compare the effectiveness of chemical defense against that of sloughing and overgrowth, as natural levels of allelopathic substances are unknown. The potentially allelopathic compounds co-extracted with the settlement inducers might primarily reduce the settlement of epibionts until the sloughing of epithallial cells of CCA surface occurs. Our combination of choice and no-choice settlement and survival experiments indicate that natural levels of allelopathy are insufficient to prevent coral settlement, that overgrowth is relatively ineffectual, and that sloughing is a highly effective strategy in preventing the survival of coral larvae on the five species of CCA tested.

Larval ranking of potential settlement substrata

We can now confirm that *T. prototypum* plays a critical role in influencing the fine-scale distribution patterns of coral recruits. Covering up to a few square centimeters, *T. prototypum* patches provide an excellent attachment surface for corals upon which competition and predation from other nonmotile reef organisms is low. However, *T. prototypum* comprises <5% of the CCA flora on GBR reefs (R. Steneck, E.

Turak, L. Harrington, and T. Done *unpublished manuscript*). So, rather than specializing in settlement exclusively onto a single uncommon CCA species, the larvae are able to actively recognize and select the next preferred substrata as suitable settlement sites, possibly keying into surface morphogens found in varying abundance or structure among CCA species (Fig. 5A). *Hydrolithon borgesenii* has been described as the key substratum for coral settlement in the Caribbean (Raimondi and Morse 2000). A similar species, *H. reinboldii*, is dominant throughout the tropical Indo-Pacific (Adey et al. 1982), and this species also contained a highly potent methanol-soluble morphogen and exhibited the second highest settlement and survival rates out of the remaining CCA species.

Habitat selection by planktonic larvae is critical to subsequent survival of sessile invertebrates, since location largely determines the environmental conditions experienced by later life stages (Keough and Downes 1982, Baird et al. 2003). Apart from recently disturbed and damaged reef surfaces, CCA offers a potentially favorable attachment site for disparate marine invertebrate taxa including coelenterates, which is stable and can facilitate survival and development to reproductive maturity. Among these settlers, the specificity of the interaction covers a spectrum, from species that manifest specificity for a particular species of CCA (Morse et al. 1996), to those requiring contact with any of a variety of CCA (Morse et al. 1988). We further demonstrated that coral larvae are able to actively recognize and rank different species of CCA as suitable settlement sites primarily based upon chemical signature and clearly reflecting the effectiveness of their antifouling strategies. This ranking process strongly supports the notion that settlement behavior (habitat selection) is adaptive and may be largely responsible for the fine-scale recruitment patterns observed in the field leading to a wider influence on coral reef structure.

Acknowledgments

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APPENDIX

A photograph showing examples of anti-settlement defenses in crustose coralline algae is available in ESA's Electronic Data Archive: *Ecological Archives* E085-120-A1.

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SCIENCE

Phonotaxis of Crickets in Flight: Attraction of Male and Female Crickets to Male Calling Songs

Abstract. *Large numbers of two species of mole crickets flew to loudspeakers playing the appropriate calling song outdoors. Mated females were more frequently captured than unmated ones, and males were 12 percent of the catch. Crickets of three other subfamilies were trapped as they flew to mole cricket songs resembling their own.*

Most male crickets and katydids (*1*) make species-specific calling sounds that enable appropriate mute females to find their way to them (*2*). Generally the unmated female walks or runs to the conspecific male. In the only reported case of flight bringing the pairs together, orientation to the conspecific sound occurred before flight (*3*). In studying the acoustic behavior of two species of mole crickets, we discovered that males and mated females as well as unmated females terminated long-range flights (*4*) by orienting to and landing near sources of conspecific calling songs.

Scapteriscus acletus (southern mole cricket) and *S. vicinus* (changa) are important agricultural pests in the southeastern United States. The males burrow in soil and produce calling songs in specially constructed chambers. When we discovered that flying mole crickets were landing near the entrance to the burrow where the male was singing, we broadcast a recorded calling song and dozens of flying mole crickets rained on the speaker. We studied this phenomenon in 1972 and 1973 near Gainesville, Florida, at a lighted golf course and an unlighted pasture. Our experimental setup consisted of two independent broadcast systems and three large metal funnels (1.2 m in diameter) placed 3 to 15 m apart (Fig. 1). Each broadcast system included a battery-operated tape recorder, a battery-operated audioamplifier, and a speaker (*5*). Each speaker was mounted in the center of a funnel and was aimed directly upward. A jar ring was soldered to the bottom of each funnel (5 cm in diameter), and the adults that flew into the funnels were collected in numbered 500-ml jars. The natural calling songs of *S. acletus* and *S. vicinus* were tape-recorded in the field, with the microphone 15 cm above ground level. The soil temperature was 25°C. Synthetic calling songs were made and tape-recorded in the laboratory (*6*). The intensity of the broadcast songs was measured 15 cm above the speaker (*7*) and was maintained at 100 ± 3 db during all experiments (*8*).

Broadcasting trials began about 0.5 hour after sunset and continued until most flight ended, about an hour later. Tests were made only when the soil temperature was 25 ± 3°C. During each trial both calls were broadcast simultaneously, and the trial was ended when at least one of the 500-ml jars contained 20 or more mole crickets. A predetermined duration was not used for each trial because the numbers of mole crickets flying varied greatly at different seasons, dates, and times. Trials in which no jar yielded as many as 20 mole crickets were disregarded. The jars with trapped mole crickets were detached from the funnels after

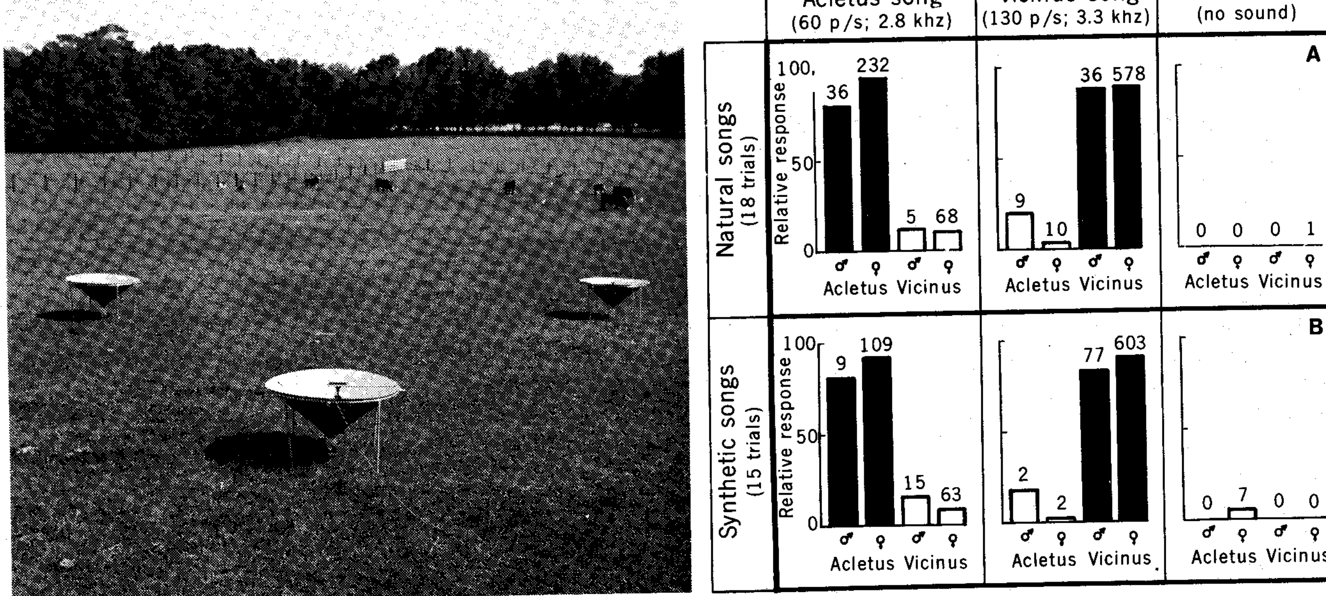

Fig. 1 (left). Sheet metal funnels used to trap mole crickets. The control trap (left back) has no speaker. Fig. 2 (right). Specificity of response of flying *Scapteriscus acletus* and *S. vicinus* to broadcast tape recordings of (A) natural and (B) synthetic calling songs. Crickets captured in traps broadcasting conspecific songs are indicated by black bars. Others are indicated by open bars. Each bar shows the percentage of the total number of a sex and species that was captured by traps during the trials with natural songs or during the trials with synthetic songs. The number of individuals is indicated above each bar; *p/s*, pulses per second.

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Table 1. Attraction of other flying crickets to broadcast songs of *Scapteriscus acletus* (60 pulses per second; 2.8 khz).

Subfamily and species	Number attracted		Calling song of attracted species (25°C)		Source of *S. acletus* song
	Male	Female	Pulses per second	Carrier frequency (khz)	
Gryllinae, *Gryllus rubens*	7	12	55	4.8	Natural and synthetic
Oecantinae, *Oecanthus celerinictus*	3	11	65	3.8	Synthetic
Nemobiinae, *Neonemobius cubensis*	0	8	55	7.3	Synthetic

each trial and replaced with empty ones.

The speaker first used to broadcast a particular song was selected randomly; in subsequent trials of the same pair of songs the speakers used were alternated in order to negate position effects. During each trial one of the three funnels served as a control (*9*). At the end of each evening's trials the mole crickets in each numbered jar were identified (*10*), sexed, and counted. For each species, on three or more occasions separated by 1 week or more, 10 to 25 of the trapped females were examined for sperm in the spermathecae.

At the golf course mole crickets could be seen flying at lights 100 m from the funnels. When broadcasting began, the crickets would alter direction and fly toward the funnels. Many dropped or flew into the funnels. Others landed on the sod nearby. At the unlighted pasture the crickets could be seen only as they neared the funnels. Trials involving playbacks of taped natural songs (Fig. 2A) showed that *S. acletus* and *S. vicinus* are principally attracted to their own songs.

Trials with synthetic songs (Fig. 2B) produced results similar to those with natural sounds, thus showing that no differences other than those in the carrier frequency and pulse rate were required to elicit the species-specific responses (*11*). For each species, with both natural and synthetic songs, a larger proportion of females than males showed conspecific responses. A significantly higher proportion (10 percent) of *vicinus* females were trapped at *acletus* songs than vice versa (3.4 percent) ($P < .01$). Of 66 *acletus* females examined, 91 percent had sperm in the spermathecae. Of 56 *vicinus* females, 61 percent had sperm.

Crickets other than mole crickets sometimes flew into the traps broadcasting *acletus* songs (Table 1). In none of the trials were such crickets captured in the silent (control) trap or in the trap broadcasting *vicinus* songs. Although the three species captured represented three subfamilies, their calling songs were similar to that of *acletus*. Specifically, the calling songs of all three were trills with pulse rates at 25°C within five pulses per second of the *acletus* trill. Males of only two of the species were captured. At least some of the *Gryllus rubens* females had mated before capture, for they subsequently laid fertile eggs.

The attraction of virgin female crickets to male calling songs is easily understood. The attraction of females with sperm in their spermathecae (*12*) and especially the attraction of males are less easily interpreted. We interpret the flights of these individuals (and perhaps of virgin females, too) as dispersive, and we suggest that they are using the sexual signaling of males of their species as an indication of a habitat suitable for colonization. We originally thought that those flying toward the sound as a habitat-indicating signal and those flying toward it as a male-indicating signal should end their flights differently. We therefore predicted that those landing within the sound trap would include a significantly higher proportion of females than those landing outside the trap. However, we compared the sex ratio of 769 *acletus* that had landed outside the trap with that of 694 that had landed inside and found no significant difference ($P = .21$). We now believe that both sexes, and mated as well as unmated females, home in on the sound with equal accuracy, but we conjecture that on landing the virgin females run to the singing male's burrow while others run and burrow elsewhere. Morris (*13*) demonstrated phonotaxis by males to male calling songs in conocephaline katydids; however, the context was male-male aggression over occupation of territory (or broadcasting space). Male-male aggression occurs in crickets and has been studied and described, but approach of males to male calling songs has not been reported (*14*). We have never observed it in earthbound mole crickets.

Attraction of large numbers of flying crickets may prove useful in control—either as a means of destroying crickets or as a means of timing control procedures. We have already put it to use in studying the flight ranges of mole crickets and in studying the features of the songs responsible for species-specific responses (*4, 11*).

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References and Notes

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4. Mark-release-recapture studies of *S. acletus* showed that some individuals fly at least 715 m (S. M. Ulagaraj, in preparation).
5. We used Nagra III and Nagra IV tape recorders during 1972 and cassette tape recorders (Bell & Howell model 294, 7235, and Sony model TC-66) during 1973. The latter were checked daily for excessive flutter and aberrant tape speed. The amplifiers were an Alton Electronic audio amplifier (1972) and a Realistic model MPA-20 (1973). The speakers were Realistic model 40-1228 covered with aluminum wire screen.
6. The electronic synthesis was similar to that described by T. J. Walker, *Ann. Entomol. Soc. Am.* **50**, 629 (1957). The pulse interval ratio was maintained at 1 : 1.
7. A General Radio model 1551-B sound level meter was used to monitor the intensity.
8. The intensity of calling males measured in the field was 42 to 92 db at 15 cm above the burrow entrance. We discovered that the catch of mole crickets in sound traps was dramatically increased by using higher than natural sound levels.
9. An empty funnel with no sound (control) generally caught no mole crickets. It never had more than 1.5 percent of the number of mole crickets in the other two traps.
10. *Scapteriscus* species can be easily identified by morphological characters [W. S. Blatchley, *Orthoptera of Northeastern America* (Nature Publishing, Indianapolis, Ind., 1920)].
11. Further experiments with synthetic sounds showed that the species-specific response of *S. acletus* includes discrimination of both pulse rate and carrier frequency (S. M. Ulagaraj and T. J. Walker, in preparation).
12. One of us (S.M.U.) has twice observed a female land and enter the burrow of a calling male. One of the two females was dug out a few minutes later and found to have sperm in the spermathecae. It is doubtful that mating was completed during the interval. We do not know whether female mole crickets mate more than once or with more than one male. Because they lay substantially fewer (but larger) eggs than most other female crickets, we doubt that multiple matings are usual.
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23 July 1973

for events occurring farther apart in time. Coallocation is not limited to linking memories at encoding. Memory recall may engage a similar process to link new with old memories. We trained mice on event 1, 2 days before event 2. Event 2 memory was enhanced if event 1 was recalled 6 hours, not 24 hours, before event 2 (Fig. 4D). Here, we find that excitatory-inhibitory balance determines whether memories are bound or, alternately, segregated in the LA. More broadly, these principles provide a foundation for understanding how memories are organized within associative networks.

Note added in proof: During final preparation of this manuscript, a notable study showing time-limited coallocation of hippocampal memory traces was published (*25*).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6297/383/suppl/DC1
Materials and Methods
Figs. S1 to S9
References (*26*–*43*)

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BEHAVIORAL ECOLOGY

Reciprocal signaling in honeyguide-human mutualism

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Greater honeyguides (*Indicator indicator*) lead human honey-hunters to wild bees' nests, in a rare example of a mutualistic foraging partnership between humans and free-living wild animals. We show experimentally that a specialized vocal sound made by Mozambican honey-hunters seeking bees' nests elicits elevated cooperative behavior from honeyguides. The production of this sound increased the probability of being guided by a honeyguide from about 33 to 66% and the overall probability of thus finding a bees' nest from 17 to 54%, as compared with other animal or human sounds of similar amplitude. These results provide experimental evidence that a wild animal in a natural setting responds adaptively to a human signal of cooperation.

In 1588, João dos Santos, a Portuguese missionary in Sofala (in present-day Mozambique), often noticed a small bird flying through cracks in the walls of his mission church and nibbling wax from the candlesticks within. This kind of bird, he wrote, had another peculiar habit of leading men to bees' nests by calling and flying from tree to tree. After the men harvested the honey, the birds would eat the wax combs left behind (*1*). We now know this species to have been the greater honeyguide *Indicator indicator* and dos Santos's description to have been accurate. Honeyguides eat beeswax and know where bees' nests are located; humans can subdue the bees and open the nest using fire and tools, thus exposing beeswax for the honeyguides and honey for the humans (*2*). This interaction remains an extremely rare example of mutualism between free-living wild animals and our own species. Here we show that it is also a specific example of reciprocal communication between birds and humans.

Greater honeyguides (Fig. 1A) seeking a human collaborator approach people and give a loud chattering call (audio S1). This call is distinct from their territorial song and is accompanied by referential gestures (*3*): the bird flies from tree to tree in the direction of the bees' nest until its human follower finds the nest (*2*, *4*). The honeyguide thus directs a signal of the bees' nest location toward humans, and the honey-hunters use this signal to their mutual advantage. In this study, we ask whether honeyguides in turn exploit specialized signals directed at them by humans. We studied greater honeyguides (hereafter "honeyguides") in the Niassa National Reserve in northern Mozambique. This region has been noted for its honey and beeswax production at least since Arab trading times centuries ago (*5*, *6*). The local Yao people still harvest wild honey using traditional methods, and this practice remains economically important.

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First, we confirmed that in northern Mozambique, honeyguides give reliable information to human honey-hunters. To test whether guiding behavior accurately indicates the direction of bees' nests and leads to their successful discovery by humans, we trailed honey-hunters following honeyguides and tracked our movements via GPS. A guiding event was defined as a bout of guiding by an individual bird, sometimes involving consecutive journeys to different bees' nests. Each guiding event probably involved a different individual honeyguide, as the study area was 230 km^2, and the home ranges of individual honeyguides that we measured using radio telemetry did not exceed 1 km^2 and overlapped with one another (*7*) (fig. S1). 75.3% of guiding events led to the successful discovery by humans of at least one bees' nest [mean ± SE = 1.00 ± 0.08 nests; range = 0 to 3 nests; n = 97 events, excluding controls in the experiment discussed below (*7*)]. 94.6% of nests shown belonged to the honeybee *Apis mellifera*, and the rest to stingless bee species (*7*). Nests were located 0 to 832 m (median = 152 m, n = 84 nests, only considering the first nest per guiding event) from the point where guiding began. Figure 1B shows that the birds' initial flight direction accurately signaled the ultimate location of the bees' nest, corroborating a classic study from Kenya (*2*). Overall, 74.5% of bees' nests found by humans (n = 149) involved the help of a honeyguide.

Second, we asked whether the signals used by human honey-hunters provide reliable information to honeyguides. Honey-hunters seeking honeyguides often announce their presence with unspecialized sounds such as shouting and chopping wood (*4*, *8*). In some parts of Africa, however, humans also make specialized vocalizations used only when hunting honey. In the Niassa National Reserve (and, more widely, in northern Mozambique and adjacent southern Tanzania), Yao honey-hunters seeking and following honeyguides produce a loud trill followed by a grunt: "brrrr-hm" [audio S1; see (*9*) for a melodious whistle used in the same context by the Hadza people of northern Tanzania]. To confirm that "brrrr-hm" is a specialized honey-hunting sound, we interviewed 20 Yao honey-hunters, all of whom reported that they used this specific sound when hunting honey but in no other context. When

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asked why, they reported that they learned it from their fathers and that it is the best way of attracting a honeyguide and maintaining its attention. Therefore, this sound has the potential to reliably signal to honeyguides that a prospective human partner is specifically seeking honey and has the tools, skills, and time to open a bees' nest, which many humans do not. A payoff to the bird reliably results from interacting with such a partner, because if a bees' nest is harvested then wax is left behind, either as combs containing no honey or as chewed lumps spat out by honey-hunters.

Finally, we examined whether honeyguides associated this vocal signal with a higher chance of a payoff from cooperation. If so, then honeyguides should be more likely to initiate collaboration with humans producing this honey-hunting sound rather than other sounds. To test this, we carried out 72 15-min experimental transects simulating honey-hunting forays, in which an author and two local honey-hunters walked while playing back one of three acoustic cues every 7 s at consistent amplitude using a calibrated speaker: (i) a control human sound (either the Yao words for "honeyguide" and "honey" or the honey-hunter's name, alternated among transects); (ii) a control animal sound (either the song or the excitement call of the ring-necked dove, *Streptopelia capicola*, alternated among transects); or (iii) the specialized "brrrr-hm" honey-hunting sound [see (7) for details and audio S2 to S4 for examples]. Each transect used a distinct playback exemplar recorded from a different individual person or bird. Honeyguides have never been confirmed to guide any species besides humans (10). We conducted this experiment during the hot dry season; the average shade air temperature at the end of each transect was 31°C (range = 23° to 38°C). Therefore, we also tested the effects of temperature, trial time relative to sunrise or sunset, and morning versus afternoon [details in (7)].

We were guided by a honeyguide on 30 of 72 transects. Transects accompanied by the honey-hunting call had a 66.7% probability of eliciting guiding from a honeyguide, which was significantly greater than that for transects accompanied by the human control sounds (25%) or animal control sounds (33.3%) (Fig. 2A; planned comparison with controls: estimate ± SE = 1.13 ± 0.38, Z = 2.96, P = 0.0031). The probability of guiding did not differ between the two control treatments (estimate ± SE = 0.25 ± 0.33, Z = 0.76, P = 0.45). The best model also included the time relative to sunrise or sunset as a covariate (probability of being guided weakly decreased closer to the middle of the day: estimate ± SE = −4.34 ± 0.20, Z = −2.13, P = 0.034) and, overall, explained 25% of the variance in probability of being led by a honeyguide.

Once a honeyguide initiated guiding behavior, we followed it while continuing to play back the acoustic treatment, while the honey-hunters searched visually for bees' nests [see (7) for measures taken to encourage and validate equal search effort]. This revealed that honeyguides tended to cease guiding behavior more often when either of the two control sounds was produced, resulting in no bees' nests being found. Of those transects on which we were led by a honeyguide, we found a bees' nest for 81.3% when accompanied by the honey-hunting sound, compared with 66.7 and 50.0% when accompanied by the human and animal control sounds, respectively. Overall, the honey-hunting sound resulted in a 54.2% predicted probability of finding a bees' nest (Fig. 2B; planned comparison with controls: estimate ± SE = 1.21 ± 0.39, Z = 3.14, P = 0.0017) compared with 16.7% for each of the control sounds (planned comparison between controls: estimate ± SE = 0.03 ± 0.39, Z = 0.08, P = 0.94). Thus, production of the honey-hunting sound more than tripled the probability of finding a bees' nest during a standardized 15-min search accompanied by an acoustic cue. This finding experimentally validates the honey-hunters' claims that the honey-hunting sound improves their foraging success.

Honeyguides might respond more to humans producing the honey-hunting sound either because they recognize and prefer it or because they are simply more likely to hear it versus control sounds. If the latter is true, then honeyguide behavior should be predicted by playback amplitude after attenuation in the environment. We measured the mean and maximum amplitudes (in units of A-weighted decibels) of every playback exemplar with a sound-level meter at a distance of 30 m in the natural habitat of these birds

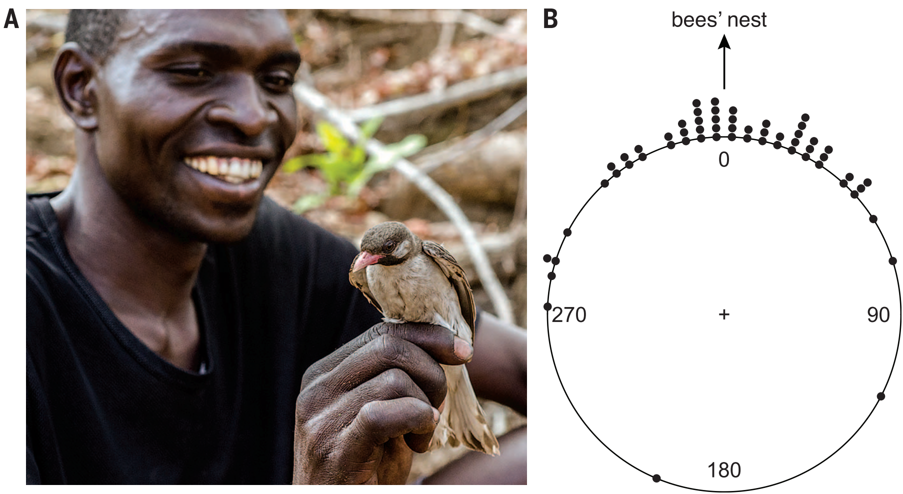

Fig. 1. Greater honeyguides accurately lead humans to bees' nests. (**A**) A Yao honey-hunter and a wild, free-living honeyguide. (This bird was captured using a researcher's mist-net and is neither tame nor habitually captive.) (**B**) Accuracy of honeyguide initial guiding behavior in relation to direction of successfully located bees' nests. Points represent the difference in bearing between initial guiding trajectory over the first 40 m of travel and the ultimate direction of the bees' nest (here set at 0) and are binned into 5° intervals. Each point represents a journey (n = 58 journeys) to a separate bees' nest that was at least 80 m away from the point where guiding began. Sometimes a honeyguide led humans to more than one nest consecutively (n = 50 guiding events). The circular distribution is unimodal (Rayleigh test, P < 0.001) with a mean of 1.7° (95% confidence interval includes zero: 352.3° to 11.1°), showing that honeyguide behavior offers reliable directional information to humans.

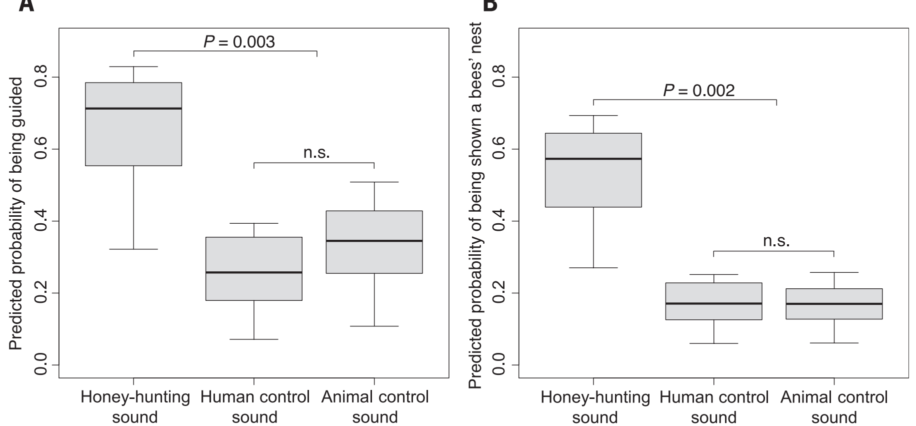

Fig. 2. Probability of a successful mutualistic interaction, in relation to experimentally given acoustic cues. Values are predicted probabilities of (**A**) being guided by a honeyguide and (**B**) being shown a bees' nest on a 15-min search, derived from a logistic model of data from experimental transects and accounting for time of day (minutes from sunrise to sunset). Boxes show medians and quartiles; whiskers show ranges (n = 24 trials per treatment group; P values show planned comparisons; n.s., not significant).

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(table S1) and tested whether the amplitudes explained any variance in guiding behavior, either in isolation or in the multivariate models above. In no case did these acoustic measures explain any variance in the probability of being guided or being shown a bees' nest (*7*). Therefore, the honeyguides' elevated response to the honey-hunting sound is unlikely to be explained by its audibility. Instead, the most parsimonious explanation is that honeyguides associate the honey-hunting sound with successful collaboration. Such partner choice should be adaptive by allowing honeyguides to improve their net benefit from interacting with humans.

These results show that a wild animal correctly attaches meaning and responds appropriately to a human signal of recruitment toward cooperative foraging, a behavior previously associated with only domestic animals, such as dogs (*11*). Although humans use many species as foraging partners, including falcons, dogs, and cormorants, these involve trained or domesticated individuals that are specifically taught to cooperate. The honeyguide-human relationship is notable in that it involves free-living wild animals whose interactions with humans have probably evolved through natural selection. To our knowledge, the only comparable relationship involves cooperation between artisanal fishermen and free-living dolphins. Several reports exist of men "calling" dolphins to hunt, starting with Pliny the Elder around 70 CE (*12*). Whether this reflects a similarly specialized communication system to that mediating the honeyguide-human mutualism in Mozambique remains unknown.

How might honeyguides acquire information about honey-hunters' signals of cooperation? Honeyguides are brood-parasitic and reared by insectivorous hosts (*4*), which suggests that their propensity to locate bees' nests and guide humans to them is likely to be innate. However, the "brrrr-hm" human signal studied here is confined to a specific geographical area, and a different cultural group living 1000 km away uses a different signal which is likely to have the same function (*9*). Local adaptation is unlikely to account for corresponding honeyguide specialization, given a lack of obvious genetic structure across its range (*13*). This implies that local refinements to guiding behavior are probably learned, which is supported anecdotally by the belief of many Yao honey-hunters that juvenile honeyguides [which have distinctive yellow plumage (*4*)] are a separate species (called "namandindi") that, despite beckoning humans in the manner of an adult honeyguide ("sego"), falls quiet in response to the honey-hunting sound. We propose that learning might occur socially from conspecifics in the vicinity of bees' nests, resulting in a local cultural tradition among honeyguides that reflects the customs of their human collaborators.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6297/387/suppl/DC1
Materials and Methods
Fig. S1
Table S1
References (*14*–*22*)
Audio S1 to S4

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PROTEIN DESIGN

Accurate design of megadalton-scale two-component icosahedral protein complexes

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Nature provides many examples of self- and co-assembling protein-based molecular machines, including icosahedral protein cages that serve as scaffolds, enzymes, and compartments for essential biochemical reactions and icosahedral virus capsids, which encapsidate and protect viral genomes and mediate entry into host cells. Inspired by these natural materials, we report the computational design and experimental characterization of co-assembling, two-component, 120-subunit icosahedral protein nanostructures with molecular weights (1.8 to 2.8 megadaltons) and dimensions (24 to 40 nanometers in diameter) comparable to those of small viral capsids. Electron microscopy, small-angle x-ray scattering, and x-ray crystallography show that 10 designs spanning three distinct icosahedral architectures form materials closely matching the design models. In vitro assembly of icosahedral complexes from independently purified components occurs rapidly, at rates comparable to those of viral capsids, and enables controlled packaging of molecular cargo through charge complementarity. The ability to design megadalton-scale materials with atomic-level accuracy and controllable assembly opens the door to a new generation of genetically programmable protein-based molecular machines.

The forms and functions of natural protein assemblies have inspired many efforts to engineer self- and co-assembling protein complexes (*1*–*24*). A common feature of these approaches, as well as the structures that inspire them, is symmetry. By repeating a small number of interactions in geometric arrangements that are consistent with the formation of regular structures, symmetry reduces the number of distinct interactions and subunits required to form higher-order assemblies (*2*, *3*, *25*). Symmetric complexes can be designed to form through self-assembly of a single type of protein subunit or co-assembly of two or more distinct types of protein subunits. Multicomponent materials possess several important advantages, including the potential to control the initiation of assembly by mixing independently prepared components. This property could allow,

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Prof or LA interview questions

(you will answer 10 Qs; chosen randomly; audiotaped; can bring papers but no notes)

1. What was the official lab assignment, what are you doing this semester?
2. Pretend I am your mom/dad. Explain to me your research in lay-terms without diluting your purpose/methods/finding.
3. What makes your project same vs different from previous publications?
4. What research paper(s) is the most influential for your project (show me)?
5. Provide brief explanations about the significance and/or importance of background literature you have used?
6. Pull out a hard copy of one of the research papers you've read for your research project and explain to me this {title/figure/abstract/methods}.
7. Pull out a hard copy of the {Ulagaraj-crickets, Fabricius-coral, Lewis-firefly, Manser-meerkats} paper and explain this {title/figure/abstract/methods}.
8. What is the difference between a hypothesis and a prediction? Examples?
9. What are the scientific and common names of the organisms you study?
10. What is your control vs. treatment(s) in your experiment, and variables?
11. Define, provide examples, what is a positive control vs. negative control?
12. How are you using: randomization, replication, sample size; in your study?
13. What are your hypotheses for your entire research project? What are the associated predictions for each hypothesis?
14. What specific data sets will you be collecting in your studies of either animal-type? (What did they do in the publication you are replicating?)
15. Describe how you will collect data for homologous human behavior study?
16. Identify relevance & purpose of finding homologous behaviors in humans?
17. How do you plan to analyze your data?
18. Can you provide information on the gene that is associated with the communication behavior? (Give specifics: located? base pairs? function?)
19. How does your gene correlate with your behavior, what pathologies would be predicted to occur if your gene gets knocked-out?
20. Imagine you are in an interview for your dream job, or for your dream med/vet/grad school, in 5 sentences, explain the research project you led this semester and impress me so much you can feel confident "you got it".

Homework (at the end, you will then do one of these; <20 minutes):

A. Here is a set of data, calculate the average and generate error bars. How do we do a statistical test to determine if the two sets are different? If you run a statistical test and the p-value is calculated to be 0.04, and your significance level is 0.05, interpret the meaning of your p-value obtained from the statistical test. Does it prove significance? Why?

B. Draw me a labeled graph that accurately represents your predicted data (what you are predicting to be the results of your experiment).

C. Using HTML expertise, code a {X} page that would look like one expected in a formal manuscript and abides by the rules of Instructions to Authors.

D. Take your smartphone and to film <10 seconds of the behavior of that {fish, student, squirrel} and then create for me an HTML page with it as a extraordinarily professional polished scientific Figure that includes a self-starting looped video figure (like seen in a Harry Potter newspaper).

Prof or LA interview questions

(you will answer 10 Qs; chosen randomly; audiotaped; can bring papers but no notes)

1. What was the official lab assignment, what are you doing this semester?
2. Pretend I am your mom/dad. Explain to me your research in lay-terms without diluting your purpose/methods/finding.
3. What makes your project same vs different from previous publications?
4. What research paper(s) is the most influential for your project (show me)?
5. Provide brief explanations about the significance and/or importance of background literature you have used?
6. Pull out a hard copy of one of the research papers you've read for your research project and explain to me this {title/figure/abstract/methods}.
7. Pull out a hard copy of the {Ulagaraj-crickets, Fabricius-coral, Lewis-firefly, Manser-meerkats} paper and explain this {title/figure/abstract/methods}.
8. What is the difference between a hypothesis and a prediction? Examples?
9. What are the scientific and common names of the organisms you study?
10. What is your control vs. treatment(s) in your experiment, and variables?
11. Define, provide examples, what is a positive control vs. negative control?
12. How are you using: randomization, replication, sample size; in your study?
13. What are your hypotheses for your entire research project? What are the associated predictions for each hypothesis?
14. What specific data sets will you be collecting in your studies of either animal-type? (What did they do in the publication you are replicating?)
15. Describe how you will collect data for homologous human behavior study?
16. Identify relevance & purpose of finding homologous behaviors in humans?
17. How do you plan to analyze your data?
18. Can you provide information on the gene that is associated with the communication behavior? (Give specifics: located? base pairs? function?)
19. How does your gene correlate with your behavior, what pathologies would be predicted to occur if your gene gets knocked-out?
20. Imagine you are in an interview for your dream job, or for your dream med/vet/grad school, in 5 sentences, explain the research project you led this semester and impress me so much you can feel confident "you got it".

Homework (at the end, you will then do one of these; <20 minutes):

A. Here is a set of data, calculate the average and generate error bars. How do we do a statistical test to determine if the two sets are different? If you run a statistical test and the p-value is calculated to be 0.04, and your significance level is 0.05, interpret the meaning of your p-value obtained from the statistical test. Does it prove significance? Why?

B. Draw me a labeled graph that accurately represents your predicted data (what you are predicting to be the results of your experiment).

C. Using HTML expertise, code a {X} page that would look like one expected in a formal manuscript and abides by the rules of Instructions to Authors.

D. Take your smartphone and to film <10 seconds of the behavior of that {fish, student, squirrel} and then create for me an HTML page with it as a extraordinarily professional polished scientific Figure that includes a self-starting looped video figure (like seen in a Harry Potter newspaper).

Film Evaluation[1] (LBC Universal Communication Rubric)

Question	Rating
1. Does this film have an appropriate and interesting TOPIC for the circumstances? (See RHH 3a, 3c-g.)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
2. Does this film have a clear, original, specific POINT (THESIS)? (See RHH 3a, 3h-l.)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
3. Does this film provide adequate SUPPORT (REASONING AND EVIDENCE) for its thesis? (See RHH 4.)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
4. Is this film well ORGANIZED so that the audience can follow its points and examples? (See RHH 3p-q)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
5. Does this film employ CLEAR, PRECISE LANGUAGE?	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
6. Is this film factually ACCURATE and also FAIR, including recognizing reasonable objections? (See RHH 4f-h.)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
7. Is this film presented in a way that is ENGAGING to the audience?	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
8. Is this film of a REASONABLE SIZE for the circumstances (10-15min)?	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
9. Does this film clearly CREDIT OTHERS when their ideas and words are used?[2] (See RHH 7.)	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|
10. Does this film utilize editing software well and show significant EFFORT, CREATIVITY and TECHNICAL clarity when viewed?	4.0 3.0 2.0 1.0 0.0 |-----|-----|-----|-----|

[1] This is adapted from a form put together by various LBC HPS faculty. Chapter and section references are to Frederick Crews, *The Random House Handbook*, sixth edition (New York: McGraw-Hill, 1992).

[2] Note that plagiarism is grounds for failure of the assignment and the course. If you're not sure what counts as plagiarism, ask early and often!

THE ELEMENTS OF A GOOD HYPOTHESIS

The American Heritage Dictionary defines hypothesis as "*a tentative explanation for an observation, phenomenon, or scientific problem that can be tested by further investigation*". A hypothesis is a possible answer to a question, from which predictions can be made and tested. There can be multiple hypotheses used to answer a single question and for each hypothesis, multiple predictions can usually be made.

The foundation for high quality, biological research is a good hypothesis. A good hypothesis is more than just an educated guess.

THE HYPOTHESIS SCORE CARD…

A good hypothesis must:

1.) explain how or why: provide a mechanism

2.) be compatible with and based upon the existing body of evidence.

3.) link an effect to a variable.

4.) state the expected effect.

5.) be testable.

6.) have at least two outcomes.

7.) have the potential to be refuted.

Hypotheses can be scored based on these elements. When considering a hypothesis, give one point for each of the elements. An *accomplished hypothesis* will have a score of 7. An *incomplete or developing hypothesis* will have a score of 5-6. A score below 5 is an *attempted hypothesis* or *not a hypothesis*. You should use this scoring procedure when developing your own hypotheses or when evaluating hypotheses of others.

Example Student Appeal

(student appealed the scoring of their answer to an exam question)

Name Removed

What is Mendel's Law of Segregation most closely associated with: Meiosis I or Anaphase I?

Claim:

- The law of segregation is most closely associated with Anaphase I.

Evidence:

- "Law of segregation states that paired chromosomes move to opposite nuclei." (Campbell Ch.3 pp36)

  Campbell, Heyer, Paradise. *Integrating Concepts in Biology*. 2014; ch.3 pp. 36

- "Homologous chromosomes separate in anaphase I…" (Campbell Ch.3 pp16)

  Campbell, Heyer, Paradise. *Integrating Concepts in Biology*. 2014; ch.3 pp. 16

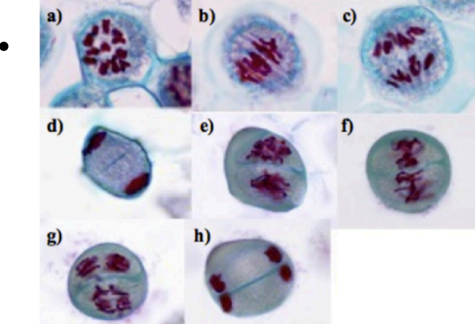
- 

  Figure 3.8 Egg formation in a lily plant with DNA stained red. (a) Parental diploid cell has undergone DNA replication. (b) Chromosomes condense and pair up with their matching chromosome. Each pair becomes closely intertwined, and then moves to the equator. (c) Paired chromosomes separate and migrate to opposite poles. (d) Cytokinesis separates the haploid nuclei. (e-f) In each cell, chromosomes are repositioned and then align on their respective equators. (g) Attached chromatids are separated and migrate to different poles. (h) Chromosomes coalesce inside nuclei and cytokinesis will produce four haploid cells.

  Figure 3.8:
  Campbell, Heyer, Paradise. *Integrating Concepts in Biology*. 2014; ch.3 pp. 15

- "The Law of Segregation states that each hereditary characteristic is controlled by two 'factors' (now called alleles), which segregate (separate) and pass into separate germ (reproductive) cells." (Hine & Martin)

  Hine, R. and E. Martin. A Dictionary of Biology. *Oxford University Press*. 6.

Reasoning:

- Meiosis I is the first half of meiosis that ends in two diploid cells, and is followed by meiosis II which ends in four haploid gametes. Gregor Mendel devised two laws to help understand this overall process of meiosis. These two laws are as follows: The Law of Segregation and The Law of Independent Assortment. In this case, we focused specifically on the law of segregation. This law is most specifically associated with anaphase I. To better illustrate this, one must understand what happens during anaphase I. In this stage, the homologous chromosomes separate and migrate to their opposite poles. As stated in our book, and in the Dictionary of Biology (published by Oxford University Press) it says that the law of segregation explains how the homologous chromosomes split and move to opposite nuclei, which is identical to the description of what happens in anaphase I. As shown by Figure 3.8 (found in Ch3 on page 15) the image labeled "C" illustrates anaphase I. In this image, the chromosomes are dyed red, which are shown splitting and moving to opposite sides of the cell. In conclusion, the Law of Segregation is specifically associated with anaphase I.

By George Edward Hyde, Esquire

<u>*Draconian Contract*</u>

- **Purpose**

  To establish contractually binding ground rules for a team if the previously existing contract generated by the group is unclear and inadequately detailed.

- **Professional Agreement**

  As a member of this Research Team, I agree to do my part of the project—based on my designated Team Role (Pg.21). In addition to doing my part, I agree to putting in an equal work effort in order to pull my weight for the team. Also, I agree to bring an outstanding work ethic to the team—so that we may achieve a 4.0 level grade in the course. Therefore, I agree to take on all of the course work that a 4.0 level grade entails. Finally, I agree to treat my teammates with the upmost respect—by uphold all terms of this binding contract.

- **Clause I -Established Meeting and Communication Expectations:** Ground rules that will set up a known time for students to meet—thus allowing them to guarantee that additional out of lab hours will be put it for this course. In addition, to set up a known communication plan that includes the means of communication that will be utilized (with the preference included). Finally, establishing time and place to meet prior to the beginning of lab.

  A. <u>Team Meeting</u>
     1. **As a team we will meet bi-weekly for laboratory.** The location of the meeting will be the East Lounge of East Holmes Hall.
        - I. Sunday – 9am to 10am
          - a. Prepare for the upcoming weeks lab.
          - b. Discuss the assigned pre-readings.
          - c. Formulate a plan for use of in lab hours.
            - i. See Appendix for Plan Worksheet
          - d. Observe Proposed Animal Communication Behavior
        - II. Friday – 6pm to 7pm
          - a. Discuss what was presented in lab that week.
            - i. Lecture topic notes
            - ii. Assignments
          - b. Gather data from Observations
            - i. Work on Manuscript and Documentary
              - Divide and Conquer using Team Roles (Pg.21)
          - c. Formulate a plan for the next meeting
            - i. See Appendix for Meeting Plan Worksheet
     2. **All members MUST attend the Team Meetings.**
        - I. In the case where a member will be unable to attend, they must contact all members 24 hours in advance.
          - a. Maximum Excused Absences = 2 meetings

II. If a member does not contact the team 24 hours in advance, or misses more than 2 meetings—**Go To Clause II.**

B. Team Communication
1. As a team we will communicate through the following means in order…
   I. Cell Phone Group Message
      a. ALL members will respond to a given message within an HOUR of message being delivered.
         i. If a member does not follow the ground rules—**Go To Clause II.**
   II. Team Email—using strictly @msu.edu
      a. ALL members will respond to a given message within an HOUR of message being delivered.
         i. If a member does not follow the ground rules—**Go To Clause II.**

C. Pre Lab Meetings
1. ALL members will show up to each scheduled lab class meeting
   I. Prior to lab beginning, ALL members will meet 10 minutes before regular class, and 15 minutes before class when important assignments are due (i.e. Manuscripts)
      a. Location = Directly Outside of C4 Holmes Hall

- **Clause II -Meeting and Communication Breach of Contract Incidents:** Ground rules to establish a known protocol concerning how the team will deal with a breach of contract. A breach of contract, by definition, is time when a binding agreement has not been honored by one or more parties involved in the agreement. When breach of contract has occurred, a statement must be filed with the LB144 teaching assistant (TA) that states how the contract was breached and who has not honored the agreement.

A. Not Attending Meetings
1. As stated in Clause IA Paragraph 2 Subset I—Any member who is not able to attend a scheduled mandatory meeting must contact his/her team 24 hours in advance. Also, any member is allowed 2, and ONLY 2, excused absences from the mandatory meetings. While Clause IC Paragraph 1 states the team will meet prior to lab.
   I. If any member fails to uphold this portion of the agreement a breach of contract has occurred.

      a. First Incident Claim
         i. If any member fails to contact the team 24 hours in advance of missing a mandatory team meeting or has missed a total of 3 team meetings or has missed a pre lab meeting, the team must declare this breach of contract BOTH in the CATME feedback surveys as well as in writing. The CATME feedback surveys will occur regularly throughout the

semester. A hard copy of written notice must be given directly to the offender(s) and a hard copy also provided to the TA in lab section. The hard copy allows the LB144 Teaching Team to date and file the initial claim for record.

- The form for breach of contract written claims can be found in the Appendix.
  - A new clam is not permitted for 2 weeks.

b. Second Incident Claim

i. If any member fails to contact the team 24 hours in advance of missing a mandatory team meeting (for the Second Time) or has missed a total of 4 team meetings or has missed a pre lab meeting, the team must declare this breach of contract BOTH in the CATME feedback surveys as well as in writing. The CATME feedback surveys will occur regularly throughout the semester. While the writing must be given directly to the offender(s) and a hard copy provided to the TA in class. The hard copy allows the LB144 Teaching Team to date and file the claim for record.

- The form for breach of contract written claims can be found in the Appendix.

ii. In addition, the team and TA must arrange a date for a Face-to-Face Encounter—in which ALL team members MUST attend.

- Failure to attend the Face-to-Face encounter will result in immediate dismissal of the member(s) from the team—**Go To Clause IV.**
  - A new claim is not permitted for 2 weeks.

c. Third (and Final) Incident Claim

i. If any member fails to contact the team 24 hours in advance of missing a mandatory team meeting (for the Third Time) or has missed a total of 5 team meetings or has missed a pre lab, the team must declare this breach of contract BOTH in the CATME feedback surveys as well as in writing. The CATME feedback surveys will occur regularly throughout the semester. While the writing must be given directly to the offender(s) and a hard copy provided to the TA in class. The hard copy allows the LB144 Teaching Team to date and file the claim for record.

- The form for breach of contract written claims can be found in the Appendix.

ii. In addition, a hard copy of the claim must be provided to the professor (Dr. Luckie). The professor hard copy must comprehensively detail all past events, which led to all prior claims, and the current event.
   - The *professor form* for breach of contract written claim can be found in the Appendix.

iii. In addition, the team must contact the professor to setup a mandatory Face-to-Face encounter—in which ALL members must attend.
   - Failure to attend the Face-to-Face encounter will result in immediate dismissal of the member(s) from the team—**Go To Clause IV.**
   - If the professor agrees with the claims…
     - Divorce Clause proceedings begin immediately and can only be completed 2 weeks after the professor meeting.
   - If the professor does not agree with the claims…
     - The team will remain with all members in good standing. Unless the team decides to initiate the Divorce clause themselves—which at that point can only be completed 4 weeks after the professor meeting.

B. Not Following Communication Norms

1. As stated in Clause IB—any member of the must communicate in a timely and respectable fashion. The time period for all response must be within an hour of the original message.

   I. If any member fails to uphold this portion of the agreement a breach of contract has occurred.

a. First Incident Claim

i. If any member fails to respond to a group message within an hour, the team must declare the breach of contract to the LB144 TA. In order for the team to successfully submit a claim they must provide a hard copy to the offender(s) and provide a hard copy to the TA, along with a screenshot proving a lack of communication, during class. The hard copy allows the LB144 Teaching Team to date and file the initial claim for record.
   - The form for breach of contract can be found in the Appendix.
     - A new claim is not permitted for 2 weeks.

b. Second Incident Claim

i. If any member fails to respond to a group message within an hour, the team must declare the breach of contract to the LB144 TA. In order for the team to successfully submit a claim they must provide a hard copy to the offender(s) and provide a hard copy to the TA, along with a screenshot proving a lack of communication, during class. The hard copy allows the LB144 Teaching Team to date and file the claim for record.
   - The form for breach of contract can be found in the Appendix.

ii. In addition, the team and TA must arrange a date for a Face-to-Face encounter—in which ALL members must attend.
   - Failure to attend the Face-to-Face Encounter will result in immediate dismissal of the member(s) from the team—**Go To Clause IV.**
     - A new clam is not permitted for 2 weeks

c. Third (and Final) Incident Claim

i. If any member fails to respond to a group message within an hour, the team must declare the breach of contract to the LB144 TA. In order for the team to successfully submit a claim they must provide a hard copy to the offenders(s) and provide a hard copy to the TA, along with a screenshot proving a lack of communication, during class. The hard copy allows the Lb144 Teaching Team to date and file the claim for record.
   - The form for breach of contract can be found in the Appendix.

ii. In addition, a hard copy of the claim must be provided to the professor (Dr. Luckie). The professor hard copy must comprehensively detail all past events, which led to all prior claims, and include all screenshots provided to the TA.
   - The *professor form* for breach of contract can be found in the Appendix.

iii. In addition, the team must contact the professor to setup a mandatory Face-to-Face encounter—in which ALL members must attend.
   - Failure to attend the Face-to-Face encounter will result in immediate dismissal of the

member(s) from the team—**Go To Clause IV.**

- If the professor agrees with the claims…
  - Divorce Clause proceedings begin immediately and can only be completed 2 weeks after the professor meeting.
- If the professor does not agree with the claims…
  - The team will remain with ALL members in good standing—in which the professor drafts a new communication clause. Unless the team decides to initiate the Divorce clause themselves—which can only be completed 4 weeks after the professor meeting.

- **Clause III- Break of Contract Grievances related to *Performance Expectations*:** Ground rules to establish a known protocol concerning how the team will deal with grievances. A grievance, by definition, is a feeling of being wronged (or treated unfairly)—in which a statement is made stating HOW one was wronged and WHY one is not satisfied with an occurrence. Thus, in LB144 a grievance can occur from the following situations listed in this clause.

A. **Performance Expectations**

1. **As stated by the Professional Agreement section of this contract—ALL members who sign this document agreed to** …
   - I. Do their part based on Team Roles (Pgs. CoursePack)
   - II. Put forth Equal Work Effort.
   - III. Work Ethic equivalent to achieve a 4.0-level grade.
     - a. Completing all work needed for a 4.0-level grade.
   - IV. Respect all terms of the contract.

2. **Failure to uphold any of the four previously stated Professional Agreements may lead to grievances within the team.**

   I. First Incident Claim

   a. If an individual feels any of the Professional Agreements are not being fulfilled, they must declare it in the CATME feedback survey, provide a written hard copy for the TA, and have an interventional meeting (5 minutes maximum) in lab. The hard copy allows the LB144 Teaching Team to date and file the initial grievance for record.

   i. A new grievance is not permitted for 2 weeks.
   - The grievance claim form can be found in the Appendix.

II. Second Incident Claim

a. If an individual feels any of the Professional Agreements are not being fulfilled, they must declare it in the CATME feedback survey, provide a written hard copy for the TA, and have an interventional meeting (5 minutes maximum) in lab. The hard copy allows the LB144 Teaching Team to date and file the initial grievance for record.
   i. A new grievance is not permitted for 2 weeks.
      - The grievance claim form can be found in the Appendix.

b. In addition, the team and TA must arrange a date for a Face-to-Face Encounter—in which ALL members must attend.
   i. Failure to attend the Face-to-Face Encounter will result in immediate dismal of the member(s) from the team—**Go To Clause IV.**
      - A new grievance is not permitted for 2 weeks.

III. Third (and Final) Incident Claim

a. If an individual feels any of the Professional Agreements are not being fulfilled, they must declare it in the CATME feedback survey, provide a written hard copy for the TA, and have an interventional meeting (5 minutes maximum) in lab. The hard copy allows the LB144 Teaching Team to date and file the initial grievance for record.
   i. The grievance claim form can be found in the Appendix

b. In addition, a hard copy of the claim must be provided to the professor (Dr. Luckie). The professor hard copy must comprehensively detail all past events, which lead to prior grievances, and the current event.
   i. The professor grievance claim form can be found in the Appendix.

c. In addition, the team and professor must arrange a date for a Face-to-Face Encounter—in which ALL members must attend.
   i. Failure to attend the Face-to-Face Encounter will result in immediate dismal of the member(s) from the team—**Go To Clause IV.**
   ii. If the Professor agrees with the claim…

- Divorce Clause begins immediately and can only be completed 2 weeks after the professor meeting.

iii. If the Professor does not agree with the claim…

- The team will remain with ALL members in good standings. Unless the team decides to initiate the Divorce Clause themselves—which can only be completed 4 weeks after the professor meeting.

❖ **Clause IV: *Prenuptial* agreement basis for "Divorce" proceedings.** In any case where a team feels that the environment is no longer suitable—and a member is "fired" or decides to leave. The intellectual property of the group must be distributed, which is outlined in this clause.

A. **QUITTING** In the case where a Team Member decides to willingly separate from their group…

1. ALL Intellectual Property is maintained within the Team.

I. Deciding to separate from your team is equivalent to deciding to separate from a company. As is the case with a corporation, all products you created are retained by the company you departed. You have no rights to those products. All you the intellectual property—including your own work is retained by the remained group members. You therefore must start the lab over from zero as a group of 1 person. You cannot plagiarize your own prior writing, use your prior citations, methods, experimental design etc.

a. In short, you must start an entirely NEW and DIFFERENT research project.

B. **FIRING** In the case where a Team Member is fired from the Team…

1. ALL Intellectual Property is maintained with the Member of the Team who was fired.

I. Deciding to fire a member is equivalent to the team as a whole deciding to separate from the individual and all of their intellectual property. The party who was fired in this case gains the rights of the corporation when all the other group members quit. The remaining group members must, therefore, start from the lab over from Square 1 and cannot use/plagiarize their own prior writing, prior citations, methods, experimental design etc

C. **MISC/OTHER** In any case of Divorce, one party maintains all intellectual property and the other party loses all intellectual property—having to start over. Depending on the week in which the divorce is finalized, all graded assignments prior to the divorce will remain. However, the party that is required to start over will have lost the opportunity to have the feedback from these assignments for their NEW research project.

1. Example
    I. A team member decides to separate from the team after the Proposal and Half-Draft of the Research manuscript. As stated previously, this team member will be giving up all rights to all intellectual property of the team. In addition, all grades for these assignments will be maintained after the divorce. However, since the individual member must start a new and entirely different research project they have lost the opportunity to receive feedback at either the Proposal and Half-Draft stage of authorship of manuscript. Therefore, the first official graded assignment under this new research project will be the Draft1 of the manuscript.

D. **ONLY SOLO** In the case of any form of divorce—any party who either separates from the group, or is fired, MUST finish the semester as an INDIVIDUAL. Forming a new group or joining a pre-existing group is NOT permitted.

Appendix

Sunday Team Meeting Plan Worksheet

Name(s): ____

Section: ____________________ Date: ________________________

Course-Pack Readings (Pages): ____________________

Reading Summary:

____

____

____

____

Observations:

____

____

____

____

Detailed Plan for Use of In-Lab Hours:

____

____

____

____

____

____

TA Signature: X_________________________________

Friday Team Meeting Plan Worksheet

Name(s): ____

Section: ____________________ Date: _______________________

Homework:

____

____

Describe Lecture Topic:

____

____

____

____

Manuscript & Documentary—What was Worked On and By Who?

____

____

____

____

Next Meeting Agenda:

____

____

____

____

TA Signature: X_________________________________

TA Breach of Contract Form

Names: ____

Offender(s): ____

Claim: _____ First _____ Second _____ Third

Reason: _____Team Meeting _____ Communication _____ Pre-Lab

Date Occurred: ____________________

Description:

____

____

____

____

____

____

Section: ________ TA: ____________________________________

Student Signature: X ____

TA Signature: X ____

Date Issued: _______________________

Next Claim Issue Date (2 weeks): ________________________

Professor Breach of Contract Form

Names: ____

Offender(s): ____

Date Occurred: ____________________ Section: __________

Description:

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Student Signature: X ____

Date Issued: _______________________

Next Claim Issue Date (2 weeks): _______________________

TA Grievance Form

Names: ____

Offender(s): ____

Claim: _____ First _____ Second _____ Third

Date Occurred: ____________________

Description:

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____

Section: ________ TA: _____________________________

Student Signature: X ____

TA Signature: X ____

Date Issued: _______________________

Next Claim Issue Date (2 weeks): ________________________

Professor Grievance Form

Names: ____

Offender(s): ____

Date Occurred: ____________________ Section: __________

Description:

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____

Student Signature: X ____

Date Issued: ______________________

Next Claim Issue Date (2 weeks): _______________________

Divorce Form

Name: ____

Divorce Type: (Please Check One)

_____ I, ______________________, willingly have decided to separate from my group.

_____ As a team, we have decided to fire ____________________________________ .

Reason:

____

____

____

____

____

____

Date Issued: ____________________ Effective Date: _________________

Signature of ALL Team Members:

X ____

X ____

X ____

X ____

Signature of TA:

X ____

Signature of Professor:

X ____

Student Laboratory Notebook