Please answer the following questions regarding a hypothetical beetle species. Be concise. Although you may look at your notes, read any papers, and search the web, ALL WORK MUST BE COMPLETED ON YOUR OWN, without discussion with ANYONE else. Note, you should be able to answer all these questions based solely on the information below and the material you learned in our class readings and discussions.

The exam is due on Thursday, February 18th, 2010 at 5pm. Please email your exam as an MS Word document (.doc) or PDF file to queitsch@u.washington.edu. Please adhere to the word limits as indicated, double-space the lines (so I can easily add comments), and use a font size no smaller than 11 point.

Introduction:

You meet some friends at Lake Washington for a swim. You notice that there are even more algae at Madrona Beach than usual and decide against swimming. Watching the shore, you recall a recent talk by new faculty member on algae-based-biofuel production. Professor Green defined three broad areas for engineering algae into the energy source of the future: fast growth, ease of harvest, and growth in low quality, salty water. You scoop up some of the fast-growing green stuff and bring it to the lab. Under the light microscope, you find that unicellular algae of an elongated shape with a large chloroplast and two flagella make up the majority of life in your lake sample. Even under microscope, the algae in your cell counter (accommodates more cells and liquid than normal cover slide) continue to divide rapidly. If you move your microscope lights, the cells use flagella-generated motility to quickly move towards the light.

You are called to the phone in a different room and you turn off microscope and lights. Upon your return, you find that your sample now contains large, non-flagellated cells and that the rapid divisions of the flagellated cells have ceased. You transfer the large, non-flagellated cells into fresh media where they form four smaller flagellated cells which continue to divide into flagellated cells. These cultures grow in a well-lit incubator. Eventually, the cells appear to flock together and large non-flagellated cells appear. As before, each large cell subsequently forms four smaller flagellated cells. What are you observing and how is it triggered? Cell density? Nutrient limitations or light? Other factors? Combined factors? How do the cells sense the trigger(s) and/or each other?

Part One: 200 words
Propose a hypothesis to explain your observations. Think mechanistically. Design experiments that would let you test your hypothesis. Be specific in your experimental designs and outcome predictions.

Part Two: 750 words
A spontaneous mutant arises in one algae culture. First, you notice that some cells have only one flagellum. Regardless of culture conditions, these cells continue to divide and only very rarely form large non-flagellated cells. In competition experiments, your mutant quickly out-compete all cells with two flagella which periodically cease to divide and form large non-flagellated cells. Your undergraduate student notices that these cells also seem far more light-sensitive then wild-type in motility assays towards light sources of different strength. Although the altered light-sensitivity confuses you, you think you found a super-biomass producing algae
and you call your mutant “TheOne”. You snap some pictures and draw some graphs and visit Professor Green.

To your dismay, Professor Green has already started to cultivate some of the fast-growing lake algae and named it “Greenworm greenardii”. On the bright side, he has also isolated some interesting mutants and knows quite a bit about the algae’s biology and utility for biofuel production. He tells you that your mutant will be tough to harvest as it no longer responds to the signal that causes the algae to flock together. You decide to join forces and investigate the pathways controlling growth, flocculation, and mating. First, you catalogue all mutants, including your own (“TheOne”).

1. “TheOne” aka Green1: recessive, divides mitotically, fails to flocculate, fails to form zygotes, highly light-sensitive, rare mating events allow for double mutant analysis, visible phenotype: has only one flagellum

2. Green2: dominant, flocculates extremely fast after only one or two mitotic divisions, forms zygotes and flagellated offspring (i.e. mates continuously), light-sensitivity like wild-type, visible phenotype: 3 flagella

3. Green3: recessive, cells do not flocculate but form zygotes when forced in close proximity, zygotes form flagellated offspring like wild-type, light-sensitivity like wild-type, visible phenotype: 4 flagella

4. Green4: recessive, divides mitotically, fails to flocculate, fails to form zygotes, light-sensitivity like wild-type, rare mating events allow for double mutant analysis, visible phenotype: 5 flagella

5. Green5: dominant, cell flocculation and zygote formation is strongly decreased, unlike wild-type rare zygotes readily form flagellated offspring in dark and light conditions, insensitive to light in motility assays, visible phenotype: 6 flagella

6. Green6: recessive, cell flocculation and zygote formation as in wild-type, zygotes fail to form flagellated offspring in the light and dark, rare offspring allows for double mutant analysis, insensitive to light in motility assays, visible phenotype: large, non-flagellated cells

Your mutants still allow mating and offspring production, albeit at a very low frequency. What does this indicate? What complications arise for the subsequent double mutant analysis? If you could not rely on rare mating events, what approach would help you to genetically analyze this pathway(s)? What complications for double mutant analysis arise from using dominant mutants? How do you create recessive mutants of Green2 and Green5? How do you confirm that these mutants are in the same gene? What phenotypes do you expect in the respective recessive mutants if the original mutant represented a gain-of-function or a loss-of-function mutant?

You decide on double mutant analysis to create a pathway(s) to explain these confusing phenotypes. Assume no complications due to escapees. Note that the visible flagellum phenotype is just a marker for your genotype and behaves additively for recessive mutants. This phenotype is unrelated to the pathway(s) that you study. Professor Green has phenotyped some double mutants already. Results are shown for an individual non-parental tetrad type (resulting in 2 cells containing the double mutant and 2 wild-type cells). For simplicity the respective wild-type cells and additional tetrads are not shown. Please fill in the “?” and predict pathway(s) that is consistent with your data and Prof. Green’s. Draw the pathway(s) and indicate the nodes corresponding to the mutants (see also Fig.1). Be specific.
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**Part Three:** 300 words
Your Greenworm isolate is sensitive to high salt in its growth medium. You plan to conduct a large mutagenesis screen to isolate salt-resistant mutants with wild-type growth rates. Prof. Green hears of your plans and advises against it – his previous screen for salt-resistant mutants with wild-type growth failed to yield any mutants. What are possible reasons for this failure? Why did he not find dominant mutants? If mutagenesis does not work for this particular trait, what alternative approach could you use to create salt-resistant algae, particularly since your model organism is small and has a very quick generation time?

While you wait for your alternative approach to yield data, you analyze water samples from Puget Sound and discover a natural variant of Greenworm that can live in salty water. What experiments will you conduct to establish that its salt-resistance is genetically based? If so, how will you determine what these genetic variants are? In the meanwhile, Prof. Green has established a draft genome sequence of the Greenworm lake strain. In your committee meeting, Debbie advises you to just sequence the Puget Sound isolate. Do you follow her advice? What may complicate this approach and how do you circumvent these complications?

**Part Four:** 500 words
Your cloning efforts reveal that the gene affected in the Green2 mutant encodes a protein with clear orthology to the alpha component of trimeric G proteins. Recall that your first allele was dominant. Assuming that your starting mutation was a gain of function, does the Galpha signal or does the Gbeta signal in this pathway? Now assume the starting mutation was a dominant negative; does the Galpha signal or does the Gbeta signal in this pathway? How would you
distinguish these possibilities? How would reverse genetics or siRNAs interference help you with this project? What other experimental approaches may help?

What are the possible functions of Green1, Green3, Green5, and Green6? Recall your double mutant analysis and pathway predictions and re-interpret in the light of your cloning data. Given your new-found knowledge, how would you engineer salt-resistant algae that grow fast (i.e. do not mate), yet flocculate in response to an environmental signal?