Questions for Thought (QfT)

As you read this paper, write down questions you have about the logic or rationale for each experiment, the method employed, and the conclusions drawn. Come up with at least three questions. Turn in your questions as homework at the beginning of class. As you read, think about the questions listed below. Try to derive an answer from the paper or by thinking logically about the process. It may help to draw a flow chart of the various experiments. Focus on Tables I and X. We will emphasize the questions in bold; the other questions are meant to help you think about each issue.

1) Introduction: What was known at that time? What was the purpose of these experiments? What kinds of cellular processes and specific proteins do you imagine might be involved in mating? What phenotypes would you predict for mutants with loss of function in these genes?

2) Materials and Methods: How did Hartwell make the mutants? Think about each step of the mutagenesis, what is occurring during each step and why he performed it. Why did he analyze mating ability at two temperatures? That is, what is the logic for making temperature-sensitive (ts) mutants?

3) Results: How many total mutants did he obtain? Is that number a lot of mutants? How many of these were ts? Would you expect that percent of temperature-sensitive mutants? Why or why not? How many genes are represented by these ts mutants? Are these genes the only ones required for mating? How would you know you had identified all the genes involved in mating?

4) Hartwell created MATα strains from his starting MATα strains (Figure 1 steps 1-3). Why did he move all the sterile mutations into a MATα background? To test whether each new mutation is an allele of a known gene, Hartwell must assay diploids for mating. Unfortunately, normal a/α diploids do not mate. To overcome this problem, he uses a trick to create diploids that are homozygous for the “a” mating type. Cryptopleurine causes loss of the CRY+ chromosome and diploidization of the cry chromosome. He uses this selection against cryptopleurine sensitivity to obtain MATa/MATa diploids that act like haploids and try to mate (Figure 1, steps 4-6). In this way he obtains diploids with which to test complementation of the ste mutations.

Using this method (Figure 1) he obtains the results in Table 1. How many alleles of each gene (on average) does he have? Why does he see such variation in allele number? Why would you want more than one allele of a gene?
5) How would you today circumvent the labor-intensive complementation analysis?

6) Table II: Why does Hartwell retest all the mutants for sterility? Doesn’t he believe his initial results? What is he actually scoring? Why didn’t he use this assay originally? Why does he test several alleles of each gene (different strains)? Why does some mating still occur?

7) Intrinsic v Extrinsic effects. Why do mixing experiments? What is the logic for performing each of these three different experiments: mixing a few wild type cells with the mutant, mixing a few mutant cells with wild type, mixing different mutant strains with each other? What are the potential outcomes and how would you interpret the possible results? Table III: What is the evidence that the defect is “cell autonomous”?

8) Morphology, agglutination, mating factor production/destruction, budding: **What is the purpose of examining all these characteristics?** Do these assays relate back to your original predictions about the kinds of cellular processes involved in mating and the types of proteins that would perform these functions? **Table X: what have we learned about the mating process?** For example, what can we predict about ste2 function?

9) Tables VIII, IX, Suppression by cdc28: What is the logic of building double mutants with cdc28? What is the result and what does it tell you about the function of these genes in the pheromone pathway? in cell cycle control?