Answers to BIR572 Exam Questions from Joel Huberman, March 6, 2005

It's summertime in Buffalo. You're feeling too hot, so you decide to take a vacation in Antarctica. There you discover a new fission yeast species, *Schizosaccharomyces frigidiae*. Since you're passionately fond of yeast genetics, you decide to carry out some preliminary characterization of this new species. Since *Schizosaccharomyces frigidiae* seems to grow very well at low temperatures, such as 0°C, you decide to try to isolate temperature-sensitive mutants that can grow at 0° but not at 25°C.

You quickly discover that *Schizosaccharomyces frigidiae* has two mating types, which you call Plus and Minus, and you discover variants of Plus and Minus cells that are heterothallic (cannot switch mating types). By mutagenizing one of the heterothallic Plus strains and then selecting for growth at 0° but not at 25°C, you isolate 26 temperature-sensitive mutants. You backcross each of these mutants several times to wild-type heterothallic Minus cells. Then you carry out complementation tests. You find that all of the mutants are recessive, and they form just seven complementation groups.

Next, you carry out tetrad analyses to find out whether the seven complementation groups correspond to seven separate genes and to create a preliminary genetic map. You cross each of the complementation groups (termed **A–G**) with each of the other complementation groups, and you score about 60 tetrads from each cross. This is hard work, because you have to check each temperature-sensitive spore colony by complementation with appropriate tester strains to find out whether it displays a mutant phenotype due to mutation in just one of the complementation groups (and, if so, which one) or due to mutation in both complementation groups. Fortunately, there's not much else to do in Antarctica, and you're relieved to be free of the heat and humidity of Buffalo in the summertime, so you're in no rush to leave.

The following table (which extends onto the next page) summarizes the results that you obtain:

Cross	PD	NPD	T	Cross	PD	NPD	T
AxB	10	12	39	**CxD**	13	12	46
AxC	40	3	12	**CxE**	14	16	35
AxD	11	10	41	**CxF**	28	4	27
AxE	29	30	5	**CxG**	11	9	41
AxF	16	5	42	**DxE**	13	12	48
AxG	12	11	41	**DxF**	10	11	39
BxC	9	8	37	**DxG**	27	6	30

Cross	PD	NPD	T	Cross	PD	NPD	T
BxD	26	5	28	**ExF**	11	13	47
BxE	12	13	49	**ExG**	9	11	41
BxF	10	9	40	**FxG**	12	10	49
BxG	63	0	0				

14. (10 points). For each of the 21 crosses in the above table, state whether the two genes involved in the cross are genetically linked to each other or not. Also state whether one or both of the genes is linked to a centromere. In cases where you think that one or both of the genes is centromere-linked, briefly state your reasons for thinking so. Please do *not* state your reasoning in cases where you think that neither gene is centromere-linked.

Answer: In the above table, linkage between the two genes in each cross is indicated when the number of NPD tetrads is much lower than the number of PD tetrads. This is true only for crosses AxC, AxF, BxD, BxG, CxF and DxG. In some of these cases, the number of T tetrads is also reduced. The greater the reduction of NPD (primarily) and T (secondarily) tetrads, the closer the two genes must be to each other.

Most of the crosses show equal numbers of PD and NPD tetrads, and the number of T tetrads is approximately 4 times the number of PD and NPD tetrads. In other words, the ratio PD:NPD:T = 1:1:4. This ratio indicates that the two genes are *not* linked to each other, and at least one of the two genes is not close to a centromere. Please remember that *both* unlinked genes must be close to a centromere in order for the tetrad ratio to be 1:1:<4. The AxE and CxE crosses show ratios that are 1:1:<4. This indicates that A is not linked to E, and C is not linked to E, but all three genes are close to their respective centromeres. Since the proportion of T tetrads is significantly lower for the AxE cross than the CxE cross, we can conclude that A is closer to its centromere than C is. Please note that we can infer centromere linkage from a reduced T frequency ***only*** when PD = NPD. If the frequency of NPD tetrads is significantly lower than the frequency of PD tetrads, then the two genes in the cross must be linked (close to each other on the same chromosome). In such cases the frequency of T tetrads is likely to be reduced—but that reduction is a consequence of linkage of the two genes rather than of linkage of either gene to its centromere.

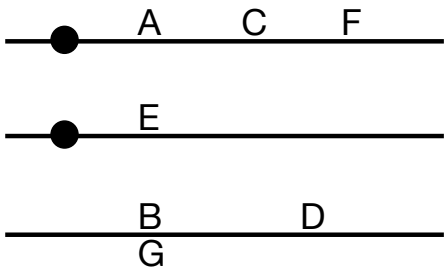
15. (5 points). Do the results suggest that each of the seven complementation groups represents a specific gene, or is there evidence for intragenic complementation?

Yes. In the BxG cross, there are no recombinant tetrads (no NPD tetrads and no T tetrads). This is the result expected when two mutations are in the same gene or in two genes that are adjacent to each other. One could distinguish between these possibilities by analyzing a much larger number of tetrads (which would have been the only option before the days of sequencing) or by determining the nucleotide sequence of the region, identifying the changes in nucleotide sequence corresponding to each mutation, and determining whether the changes occur in the same or different genes. If the two mutations were to prove to be in the same gene, then this would be an example of intragenic complementation.

16. (15 points). Based on the results in the above table, please create a preliminary genetic map of *Schizosaccharomyces frigidiae*. On this map, indicate the genes that are linked to centromeres.

Also indicate which genes are linked to each other, and indicate the order of those genes along their chromosomes. Also indicate which genes, if any, are *not* linked to any other of the tested genes. If multiple genes are linked to the same centromere, indicate which are closest to and which farthest from the centromere. **Hint: the mapping formula that we learned in class is *not* relevant to this problem; you do not need to calculate map distances; the answers to most of the parts of this question should be obvious from your responses to question 1.**

Based on the results in the table, one can conclude unambiguously that the complementation groups **A**–**G** are arranged in three linkage groups, only two of which are close to centromeres:



This map is consistent with the linkage results in the above table. In the case of the first linkage group, the frequencies of NPD and T tetrads are consistent with the order indicated (A – C – F), because there are fewer NPD and T tetrads in the crosses AxC and CxF than in the cross AxF. In the case of the third linkage group, there were no recombinants in the cross BxG, so these two complementation groups are at identical or nearly identical chromosomal positions. Furthermore, the frequency of NPD and T tetrads was about the same for the BxD cross as for the DxG cross, which is also consistent with B and G being close to each other (or equally distant from D).

If you have any further questions about these exam questions and their answers, please feel free to contact me (extension 3047; huberman@buffalo.edu).