Performing a testcross in FlyLab for two mutant genes of the fruit fly, *Drosophila melanogaster*, that are linked to calculate the recombinant frequency (RF) and estimate their relative map distance and calculate RF to estimate the relative map distance between a mutant gene and its centromere. Also determine if two mutant genes are linked or unlinked in the fungus, *Sordaria fimicola*.

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Lab demos
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Introduction:

Part 1: Recombination, Linkage and Mapping of genes in *Drosophila*

In this lab we perform a testcross using FlyLab between an F1 female with a male possessing both of the assigned parental mutations located on different genes, but are found on the same chromosome.

Question: After preforming a testcross what will be the probability of the offspring possessing the result of recombination?

Hypothesis: Because the probability of genetic recombination at any location along the chromosome is small, the number of offspring that possess the phenotypes resulting from recombination will be smaller. Will follow Mendel's law of segregation.

Assumptions: That both parent flies have different lethal mutations. The parents are true-breeding individuals (pure-bred). That the two genes are not sex-linked and one gene does not exert an influence on the other gene.

Test Cross:
♀ ++ x ♂ RI,SS (♀RI+,SS+ x ♂ RIRI, SSSS)

RI=radius incomplete wings SS=spineless bristles

	♂RI,SS
♀RI,SS	RIRI, SSSS
♀RI+	RIRI,SS+
♀+,SS	RI+,SSSS
♀++	RI+,SS+

1) The two predicted parental (non-recombinant) genotypes and phenotypes are: RI, + (RIRI, SS+) and +, SS (RI+, SSSS)

2) The two predicted recombinant (non-*parental) genotypes and phenotypes are: RI, SS (RIRI, SSSS) and +, + (RI+, SA+)

	Phenotype	Genotype	Offspring
parental (non-recombinant)	SS	SS SS	4545
	RI	RI RI	4474
recombinant (non-*parental)	SS,RI	SS,RI SS,RI	537
	+	++	505
		Total Offspring	10061

Part 2: Recombination, Linkage and Mapping of genes in *Sordaria fimicola*

Experiment 1: Assigned cross between wild-type (wt) and mutant gray (gr)

S. fimicola produces unique sac-like structures called asci , which each contains all of the haploid (n) products (ascospores) from a single round of meiosis. Like other organisms, *S. fimicola* exhibits genetic variants that result in different phenotypes that are different from the common (wild-type) phenotype. One character that exhibits several different variant types is the color of the ascospores. The common wild-type strain (wt) has black ascospores (B), but strains also can have the mutant gray strain (gr) that has gray ascospores (G) and the mutant tan strain (tn) that has tan (light-brown) ascospores (T).

Question: What will be the relative map distance between the mutant gray (gr) or tan (tn) gene and its centromere?

Hypothesis: Based on if there is recombination or not the ratios of the various coloured asci will vary.

Predictions: If there is NO crossover (no recombination) then octads carrying this configuration of ascospores are said to display a first-division segregation (MI) pattern with 4:4 ratios: 4B:4G/T OR 4G/T:4B

Experiment 2: Assigned cross between the two mutant gray (gr) and tan (tn) strains

Question: Identify and count the number of Parental Ditype (PD), NonParental Ditype (NPD) and Tetratype (TT) hybrid octads from the cross between the mutant gray strain (gr) and mutant tan (tn) strain, determine if they are linked or unlinked?

Hypothesis: Based on if there is recombination or not the ratios of the various coloured asci will vary. We apply Mendel's two principles of inheritance and do an octad analysis in order to determine if the mutant gray gene and tan genes are: linked genes (loci are physically located on the same chromosome and are sufficiently close together so that they don't assort independently). If they are unlinked genes (loci are on different chromosomes or on the same chromosome, but very far apart from each other) they assort independently of each other

Predictions: Parental Ditype (PD): octads showing the two parental (nonrecombinant) genotypes/phenotypes (gr, + and +, tn) in 4:4 ratios (4G:4T OR 4T:4G). Non-Parental Ditype (NPD): octads showing the two recombinant (non-parental) genotypes/phenotypes (+, + and gr, tn) in 4:4 ratios (4B:4C OR 4C:4B). Tetratype (TT): octads showing either two-colored ascospores (G and T only OR B and C only) with the two genotypes/phenotypes arranged in 2:2:2:2 ratios or 2:4:2 ratios OR four-colored ascospores (B, G, T and C) with the four genotypes/phenotypes arranged in 2:2:2:2 ratios.

When looking at the various strains in the lab the normal black (B) ascospores are produced only when the wild-type allele (+) is present at the loci of both genes (genotype is +, +). Mutant gray (G) ascospores are produced when the gr allele is present at the loci of the mutant gr gene, but the + allele is present at the loci of the tn gene (genotype is gr, +). Mutant tan (T) ascospores are produced when the + allele is present at the loci of the mutant gr gene, but the tn allele is present at the loci of the tn gene (genotype is +, tn). Mutant colorless (C) ascospores are produced when the gr allele is present at the loci of the mutant gr gene and the tn allele is present at the loci of the tn gene (genotype is gr, tn).

Results:

Table 1. test cross in Part 1: Recombination, Linkage and Mapping of genes in *Drosophila*

	Phenotype	Genotype	Offspring
parental (non-recombinant)	SS	SS SS	4545
	RI	RI RI	4474
recombinant (non-*parental)	SS,RI	SS,RI SS,RI	537
	+	++	505
		Total Offspring	10061
% Rf= 10.36%			

Table 2. assigned cross in Recombination, Linkage and Mapping of genes in *Sordaria fimicola*, experiment 1: Assigned cross between wild-type (wt) and mutant gray (gr) Number of MI and MII octads counted from assigned cross (wt x gr).

Assigned cross (Wt X Gr)	**Nb. of MI octads (4:4 ratios)**	**Nb. of MII octads (2:2:2:2 and 2:4:2 ratios)**
Total Number	53	60
Total Rf(%)	46.90%	53.10%

Table 3. assigned cross in Recombination, Linkage and Mapping of genes in *Sordaria fimicola*, experiment 2: Assigned cross between the two mutant gray (gr) and tan (tn) strains. Number of PD, NPD and TT asci counted from cross with mutant gray and tan strains (gr x tn).

Assigned cross (gr x tn)	**Nb. of PD octads (4G:4T or 4T:4G)**	**Nb. of NPD octads (4B:4C or 4C:4B)**	**Nb. of TT octads (2:2:2:2 ratios in two- or four-colored spores and 2:4:2 ratios in two-colored spores (B and C or G and T ONLY)**
Total Number	37	39	51
Chi-square value (X^2)	0.46	0.025	
Degrees of freedom (DF)	1	1	
Probability (p)	0.05	0.05	
Hypothesis	Accept	Accept	

Discussion

Part 1. Recombination, Linkage and Mapping of genes in *Drosophila*

1. We do a two point cross to determine the relative numbers of parental recombinant genes. Linkage map distance is calculated using the two point cross, by calculating the percentage of the offspring showing signs of recombination between the two genes that we are questioning (Cheng, 2001).

2. It can only max out at 50% recombination because recombination frequency will be 50% when two genes are located on different chromosomes or when they are widely separated on the same chromosome. This is a consequence of independent assortment, Mendel's second law. When two genes are close together on the same chromosome, they are linked since they do not sort independently. But genes located on different chromosomes will assort independently and have a recombination frequency of 50%, linked genes have a recombination frequency that is less than 50% (Hama, 1990)

In part of the lab we saw that the parental non-recombinant genotypes for the offspring were SS, SS or RI,RI, these genotypes were found in 4545 and 4474 individuals out of a possible 10061. The recombinant non-parental genes had a genotype of SSRI, SSRI and ++, and found in 537 and 505 individuals of the population. By accepting our hypothesis in this part of the lab we are able to conclude that these genes follow Mendel's law of independent assortment. The RF value in percent was found to be 10.3%, this is a reasonable number as we have learned that genes with a recombinant frequency will be less than 50%.

Part 2. Recombination, Linkage and Mapping of genes in *Sordaria*

Experiment 1: Assigned cross wt x gr

The number of MI octads which had a 4:4 ratios was 53 and the number of MII octads which 2:2:2:2 and 2:4:2 ratios was 60. The RF value of the MI octads was 46.9% and the RF value for the MII octads was 53.10%. as we have learned that gene linkage maxes out at 50%, looking at our results and keeping that in mind we know that the MI octads show signs of gene linkage as their RF value is less than 50% whereas the Rf value of the MII octads show signs of the genes being unlinked as their RF value was higher than 50%.

Experiment 2: Assigned cross gr x tn

The number of PD octads which have the ratios of 4G:4T or 4T:4G had a total amount tallied at 37. The chi-squared value for these was 0.46 with degrees of freedom being 2-1=1. The probability (p) for the PD octads was 0.05, and based on our results we therefore accepted the hypothesis. The number of NPD octads with ratios of 4B:4C or 4C:4B was tallied to be 39. The chi squared analysis proved to be 0.025, with degrees of freedom being 2-1=1. The probability for this group was 0.05 and thus our hypothesis was accepted. With both hypotheses being accepted we can conclude that these genes follow Mendel's law of independent assortment. The number of TT octads that have the ratios 2:2:2:2 ratios in two- or four-colored spores and 2:4:2 ratios in two-colored spores (B and C or G and T only) was tallied to be 51.

Overall conclusions were that the Rf value for part one of the experiments was 10.3%, which is less than the max 50% hence proving that those genes were in fact recombinant. We also accepted both hypotheses were part two of the lab meaning those genes follow Mendel's law of independent segregation.

Appendix

1. Finding RF(%) in Part 1:

RF (in %) = (Total number of recombinant offspring/Total number of offspring) X 100

RF= (537+505/ 10061)X100

RF= 10.36%

2. Finding RF(%) in Part 2, Experiment 1:

RF (in %) = (Total number of recombinant offspring/Total number of offspring) X 100

RF=(53/113)X100

RF= 46.9%

3. Calculation of Chi test for mutant Gr and Tn genes

$$X^2 = \sum \frac{(Obs - Exp)^2}{Exp}$$

Phenotype	PD Octads	NPD Octads	Total
Observed Value	37	39	76
Expected Value	40	40	77
Obs-Exp	3	1	…
$(Obs-Exp)^2$	9	1	…
$(Obs-Exp)^2/Exp$	0.43	0.025	X^{2a} =0.455

[a] Values based on the degrees of freedom 2-1=1, p value= 0.05 and the fact that the hypothesis was accepted.

References

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Hama, C., Ali, Z., & Kornberg, T. B. (1990). Region-specific recombination and expression are directed by portions of the Drosophila engrailed promoter.*Genes & Development*, *4*(7), 1079-1093.