

1. Describe the series of steps that you would perform to isolate arginine-requiring mutants from a wild-type haploid yeast strain.

i. mutagenize yeast cells.

ii. plate out mutagenized yeast cells on complete plates.

iii. replica plate colonies onto plates lacking arginine.

iiii. select colonies that grow on complete plates but not on arginine-deficient plates.

2. From problem 1 you identified 8 arginine-requiring mutants (*arg*) from an **a** mating type and 8 *arg* mutants from an α mating type. All of the *arg* mutations are recessive. You cross all the **a** mating type mutants to all of the α mating type mutants and analyze the resulting diploids for growth on plates lacking arginine. The results are shown below, where “+” means growth without arginine and “-“ means no growth without arginine.

	α 9	α 10	α 11	α 12	α 13	α 14	α 15	α 16
a 1	+	+	-	-	+	+	+	+
a 2	-	-	+	+	+	-	-	+
a 3	+	+	+	+	+	+	+	+
a 4	-	-	+	+	+	-	-	+
a 5	+	+	-	-	+	+	+	+
a 6	+	+	+	+	+	+	+	-
a 7	+	+	+	+	-	+	+	+
a 8	+	+	+	+	-	+	+	+

2a. How many different genes did you find among your mutations?

5

2b. Which mutations affect which genes? (Come up with a naming scheme for the genes and assign the numbered alleles to them.)

1, 5, 11, 12 affect the ARG1 gene; 2, 4, 9, 10, 14, 15 affect the ARG2 gene; 3 affects the ARG3 gene; 6, 16 affect the ARG4 gene; 7, 8, 13 affect the ARG5 gene.

3. You are studying aging in fruit flies and have generated a number of homozygous long-lived fly mutants. You now wish to determine how many genes these six mutants represent and you perform pairwise crosses with all of the homozygous mutants. Results of this analysis are shown in the table below (where the intersection represents the phenotype of the offspring resulting from a particular cross):

	Mut 1	Mut 2	Mut 3	Mut 4	Mut 5	Mut 6	WT
Mut 1	-	+	-	-	+	+	+
Mut 2		-	+	+	+	-	+
Mut 3			-	-	+	+	+
Mut 4				-	+	+	+
Mut 5					-	+	+
Mut 6						-	+

+ indicates all offspring have normal lifespan.

- indicates all offspring are long-lived.

WT = a wild type strain of flies.

3a. Are these mutations dominant or recessive? How do you know?

They are all recessive to WT because they have a WT phenotype when heterozygous with the WT allele.

3b. How many complementation groups do these mutations represent?

Three

3c. Describe which mutations fall into each complementation group.

Complementation group 1: 1, 3, 4; group 2: 2, 6; group 3: 5.

In addition to the long-lived fly mutants described above, you have also isolated two additional long-lived mutants (Mut 7 and Mut 8) that exhibit an unusual complementation pattern with respect to your other long-lived mutants, as shown below:

	Mut 1	Mut 2	Mut 3	Mut 4	Mut 5	Mut 6	WT
Mut 7	-	-	-	-	-	-	-
Mut 8	+	-	+	+	-	-	+

+ indicates all offspring have normal lifespan.

- indicates all offspring are long-lived.

WT = a wild type strain of flies.

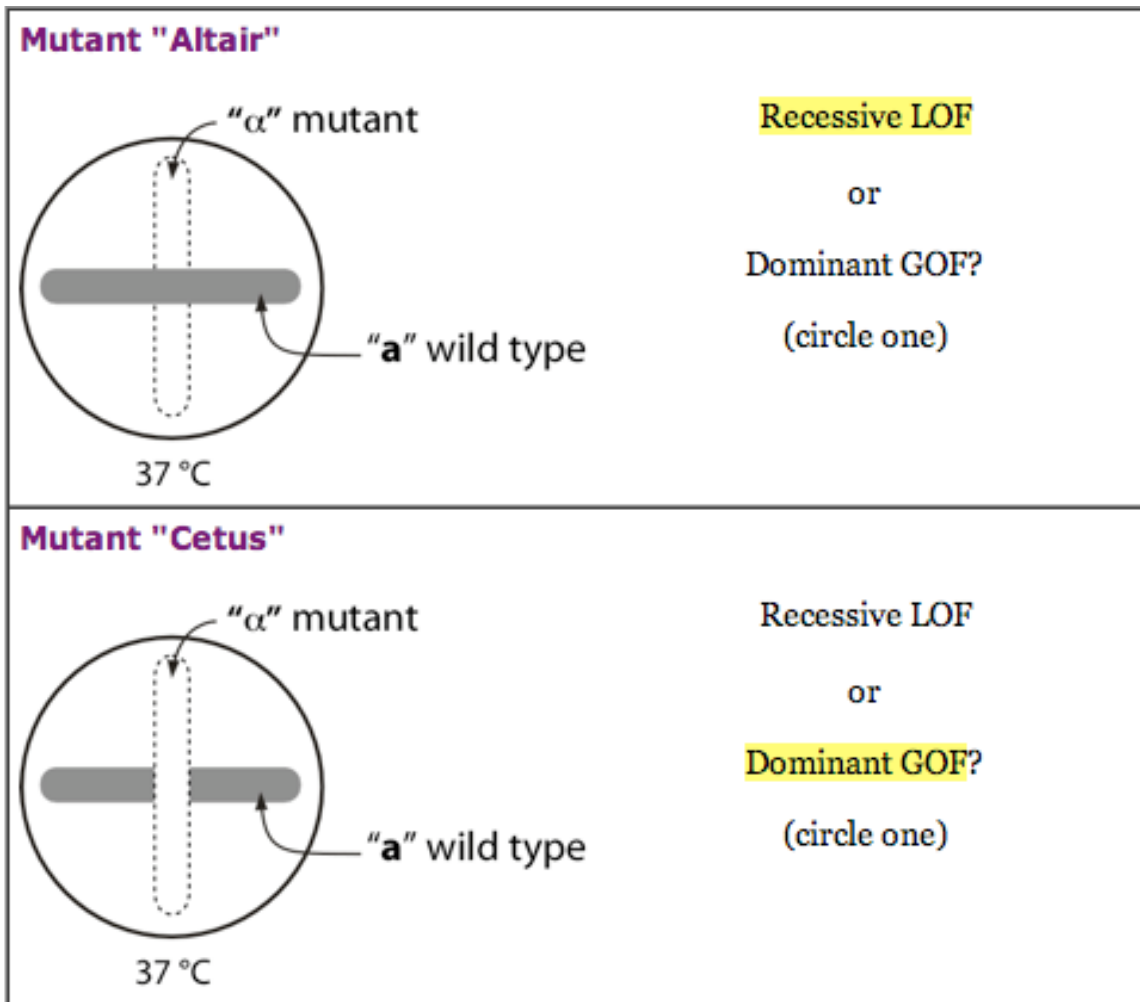
3d. What can you conclude from the results of crosses with Mut 7 and Mut 8?

Mut 7 is a dominant mutation; Mut 8 has two mutations, one in group 2 (Mut 2 & Mut 6) and one in group 3 (Mut 5).

4. Several temperature-sensitive mutant yeast strains have been isolated (named "Altair", "Cetus", "Corvus", and "Orion"). These haploid yeast strains all grow normally at 23 °C

but at 37 °C, they all show premature separation of sister chromatids following DNA replication.

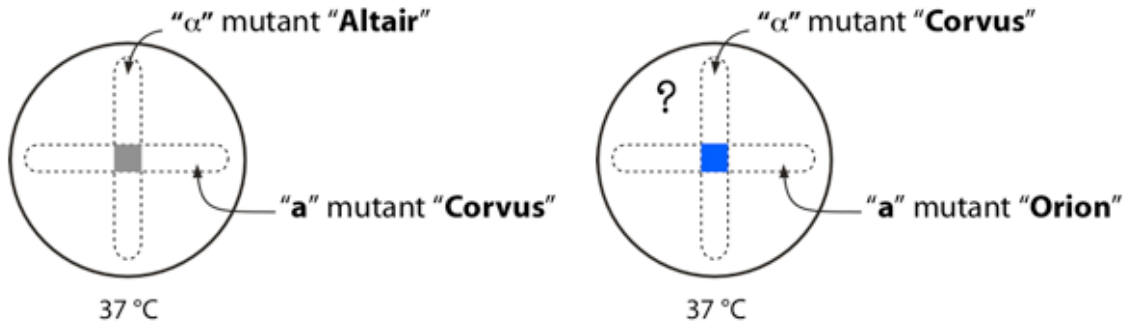
(a) Each mutant was tested as follows: mutant cells of mating type "a" were cross-stamped on a "complete" plate with wild type "a" mating type cells and tested for growth at 37 °C. The results are depicted below (where shading within each plate indicates growth). State whether each mutation is behaving as recessive loss-of-function or dominant gain-of-function, and explain how you know:



What led you to your conclusions?

The phenotype of the diploid (at the intersection of the two haploids) tells us whether the mutant phenotype is dominant or recessive. The wild haploid can grow at 37 °, so if the diploid is able to grow, it means that the mutant phenotype (no-growth) is recessive to wild type (growth). Conversely, if the diploid does not grow at 37 °, the mutant phenotype must be dominant.

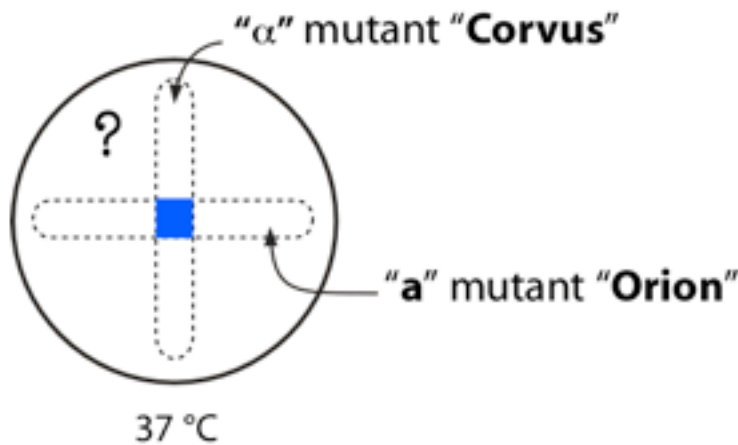
(b)



In a new test, Altair was cross-stamped with "Corvus" or with "Orion" as shown. What do you conclude from these data? [Assume that Corvus and Orion gave exactly the same results as Altair when tested as in question 3(a).]

Altair and Corvus show complementation -- i.e., the diploid made by mating these two is able to grow at 37° -- so they must be mutated in different genes such that the diploid has a working (wild type) copy of each gene. Altair and Orion do not complement, so their mutations must both be in the same gene -- the diploid is defective in both copies of one gene, so it is unable to grow at 37°C.

Predict the result of a cross between "Corvus" and "Orion" at 37 °C by shading where appropriate and if necessary in the diagram below:



From the conclusions above, we can predict that if Orion and Corvus are mated, they will be able to grow (i.e., they should show complementation):

(c) Mutants Altair and Corvus were mated to each other to form diploid cells, which were then allowed to undergo meiosis and form spores. Give the relevant genotypes of Altair and Corvus, the diploid, and the genotypes expected for the resulting spores. Use **E/e** as allele designations if you think both strains are mutated in the same gene. Use **E/e** and **F/f** as allele designations and assume independent assortment if you think the two strains

are mutated in different genes.

Altair and Corvus are both haploids, so they'll only have one copy of each gene; the same with the spores, which are the haploid products of meiosis. Since Altair and Corvus have mutations in different genes, what is mutated (recessive) in one strain will be wild type (dominant) in the other strain, and vice versa.

<p>Altair's genotype:</p> <p>$e \underline{F}$</p>	<p>Diploid's genotype:</p> <p>$\underline{E}e \underline{F}f$</p>	<p>Spore genotypes:</p> <p>$\underline{E} \underline{F}$</p>
<p>Corvus' genotype:</p> <p>$\underline{E} f$</p>		<p>$\underline{E} f$</p> <p>$e \underline{F}$</p> <p>$e f$</p>

5. There are seven genes in yeast involved in synthesizing the amino acid histidine, called HIS1 through HIS7. To keep things simple, consider loss of function mutations affecting three of the genes, HIS1, HIS2, and HIS3. You have isolated mutations in each gene as follows:

HIS1 alleles: h1, h3, h6

HIS2 alleles: h2, h4

HIS3 allele: h5

Fill in the following phenotype table, which represents complementation tests for crosses among all of these mutations:

	h1	h2	h3	h4	h5	h6
h1	-	+	-	+	+	-
h2	+	-	+	-	+	+
h3	-	+	-	+	+	-
h4	+	-	+	-	+	+
h5	+	+	+	+	-	+
h6	-	+	-	+	+	-