

Analysis of recessive mutations

STEPS TO CHARACTERIZING A RECESSIVE MUTATION

- 1) **Carry out allelism tests to determine if you have new mutation or an old friend.**
 - some alleles may not look anything like other alleles of the same gene
- 2) **Analyze expressivity and penetrance in various genetic backgrounds.**
 - expressivity: The degree to which a phenotype is expressed (on an individual that is expressing it).
 - penetrance: The percentage of individuals who express a mutant phenotype.
- 3) **Observe and document the phenotype.**
 - pleiotropy: a single gene affects more than one characteristic in the phenotype.
This is a very subjective term and can be applied to most mutants if they are carefully analyzed.
- 4) **Make efforts to obtain a null allele**
 - option 1 - look at many alleles
 - option 2 - use transposons or T-DNA
 - a null mutation will usually require molecular verification by sequencing.
- 5) **Map the gene.**
 - Arabidopsis or other sequenced genomes: use BLAST
 - Maize and other RFLP-mapped genomes: restriction fragment or PCR polymorphisms
- 6) **Make double mutants to discover how the mutants interact with other similar mutations.**
 - i) Most straightforward with recessive mutations
 - ii) Must be done in a pairwise manner. Usually, two mutations are crossed together to make the double heterozygote, and this plant is selfed. The resulting progeny will segregate both types of single mutants and double mutants. There is no way to definitely identify the double mutants in the progeny without performing allelism tests (how would you do that?)
 - iii) The interpretation of double mutant phenotypes can be complicated when regulatory genes are involved (see below).
 - Structural Vs. regulatory genes, define:
 - structural genes are “housekeeping genes” that catalyze a reaction or signaling process
 - regulatory genes control the expression of other genes. Regulators can operate at the *transcriptional* or *posttranscriptional* (protein) level and can be *positive* or *negative*

iv) There are two basic outcomes (although it is sometimes hard to tell which outcome occurred)

a) *additive phenotypes* (both phenotypes are visible in the double mutants) - two different pathways involved in forming the same structure/product have been identified

b) *epistasis*- indicates the genes operate in the same pathway

Epistasis

1) The epistatic gene is upstream in a simple biochemical pathway

hypothetical example where pairing produces a signal that activates a recombination enzyme

$cco \Rightarrow ren \Rightarrow \text{chiasmata}$
chrom. condensation recombination enzyme

cco - no condensation

ren - no recombination

cco, ren double mutant - no condensation

2) Cannot infer the order of function if a leaky allele is involved.

hypothetical example of purple pigmentation in a plant induced by UV light.
uvs is a positive regulatory gene product, *pig* the structural gene

wild type UV light $\Rightarrow uvs^+ \Rightarrow pig^+ \Rightarrow$ pigment

single mutant, *pig^{null}* UV light $\Rightarrow uvs^+ \Rightarrow pig^{null} \Rightarrow$ no pigment

single mutant, leaky *uvs* UV light $\Rightarrow uvs^{low} \Rightarrow pig^+ \Rightarrow$ low pigment

double mutant UV light $\Rightarrow uvs^{low} \Rightarrow pig^{null} \Rightarrow$ no pigment

We would erroneously conclude *pig* was first in the pathway.

3) The epistatic gene is DOWNSTREAM when a negative regulator is present.

Hypothetical example involving lignin biosynthesis in fescue. It is regulated by the pathway shown below:

$rep \text{ —| } lig \Rightarrow$ more lignin

Where *rep* is a repressor of transcription of the *lig* biosynthetic enzyme.

-the *rep* mutation causes very stiff leaf blades that break easily.

-the *lig* mutation causes weak leaf blades that do not spring back when stepped on.

-the *rep lig* double mutant is identical to *lig* (because *rep* has no function unless *lig* is present). The standard interpretation would place *lig* first, which would be in error.

Generally speaking it is very difficult to know if you have a null allele without good molecular evidence, and very difficult to know whether or not negative regulators are involved without good biochemical evidence. In many cases it may be best to assign genes to the same pathway in an unordered way (a hypothesis), and then refine the pathway using more molecular approaches.

Genetic redundancy.

a/a and b/b have ostensibly the same phenotype. This can be because of genetic redundancy (multiple genes) or biochemical redundancy (several different pathways can be used to make the same product)

When:

a ⇒ defect A (can be no defect)

b ⇒ defect B (can be no defect)

a , b ⇒ more severe defect

-most accurate when working with null mutations

-must have a clear idea that the two mutations affect the same process (e.g. two sick mutants may just get sicker when combined with each other)

Examples:

Mike Scanlon works with a gene called narrow leaf. When he selfed an F1, mutant plants were observed at a 1/16 ratio. Mutations at two loci are required to see the phenotype. He now thinks that the mutations are duplicate forms of the same transcription factor.

Many examples of genetic redundancy began with a chance gene duplication event. The two genes may then be selected for in various ways:

i) selected cumulative function. 5S RNA genes.

ii) selected for increased fidelity. e.g. chromosome segregation.

iii) selected for divergent functions but the functions are close enough that they still overlap