

Restoring the American Chestnut

How to Make a Linkage Map

Independent assortment occurs when genes/ chromosomes separate from each other independently during meiosis and therefore are inherited separately from each other. This is true if the genes for the observed phenotypes are found on different chromosomes or separated by large distances on the same chromosome so that recombination occurs greater than or equal to 50% of the time. When genes occur on the same chromosome, they are usually inherited together or are “linked.” The relative distance (not an actual measurable distance) between two genes can be calculated by observing offspring whose ratio of phenotypes do not show the expected outcome from a standard Punnett Square and finding the percentage of each phenotypic possibility. The higher the percentage that does not show both traits, the further apart on the chromosome they are.

Example using traits of American chestnut trees: (Some of the traits are depicted on the last page)

Mating: HhMm x hhmm

Where: H = Hairy leaves

h = Non-hairy leaves

M = Male sterile flowers

m = Male fertile flowers

Perform the cross:

How do you tell your genes are linked?

Restoring the American Chestnut

If the genes were independent, using standard genetic techniques you would predict that your offspring would have the phenotypic ratio (if 2000 offspring are produced):

500 Hairy/ Sterile : 500 Non-hairy/ Fertile : 500 Hairy/ Fertile : 500 Non-hairy/ Sterile

Say you make this mating, and your actual results look like this:

Hairy/ Sterile - 850	Hairy/ Fertile - 150
Non-hairy/ Fertile - 150	Non-hairy/ Sterile - 850

Obviously not 500 of each, and not close enough to be explained by chance variation therefore, the genes must be linked somehow. The location of the genes on the chromosomes will determine how often the traits will be inherited together. The closer they are, the more often the traits show up together. The farther apart two genes are from each other, the more often genetic recombination can occur during Prophase I of meiosis.

Define recombination:

How do you calculate linkage map distance?

One linkage map unit (LMU) is 1% recombination. In this case, we have a total of 300 recombinant trees (represented by the smaller numbers in the population of offspring) out of 2000 total offspring. Map distance is calculated as (# Recombinants)/(Total offspring) X 100. So our map distance is $(300/2000) \times 100$, or 15 LMU. Linkage map units do not correspond to any fixed distance on the chromosome.

Let's represent this distance in mm on the following line. (The line symbolizes a stretched out chromosome):

$x^{\text{hairiness}}$

Now let's consider another trial: (Example 2)

M = Male sterile flowers	E = Early budding
m = Male fertile flowers	e = Late budding

MmEe is mated to mmee.

Offspring:	Sterile/ early- 200	Sterile/ late- 600
	Fertile/ early- 600	Fertile/ late- 200

Restoring the American Chestnut

Again, the existence of linkage is obvious. If there were no linkage, all phenotypic classes should be about equal in number.

Find the distance in linkage map units of the recombinants (recombinants/ total) x 100:

So far we have a map that show the distance between leaf "hairiness" and flower fertility. Which side of the chestnut size gene does the leaf budding gene belong? In order to figure that out we need to look at the crosses that examine leaf hairiness and leaf budding.

(Example 3) Mating: HhEe x hhee

Offspring:

Hairy/ Early-1120	Hairy/ late-150
Non-hairy/ early-100	Non-hairy/ late- 1130

Find the distance in linkage map units of the recombinants (recombinants/ total) x 100:

Now that you have these three distances, represent the location of the genes on the line (chromosome) below:

x^{hairiness}

Do the above maps show the exact number of DNA bases in between each gene?

Do the maps show how many bases or which types of bases are involved in a particular gene?

Finally, using the following data, build a chromosome map:

R = red stems

L = long male flowers

W = wide stipules

R = green stems

l = short male flowers

w = narrow stipules

RrLl x rrlL: 124 Red/ Long

LIWw x llww: 1000 Long/ wide

RrWw x rrww: 180 Red/ wide

Restoring the American Chestnut

780 Green/ short

250 Short/ narrow

2375 Green/ narrow

110 Red/ Short

1000 Long/ narrow

170 Red/ narrow

786 Green/ long

250 Short/ wide

2275 Green/ wide

Calculate distance in linkage map units of the recombinants (recombinants/ total) x 100 for Color/ Length:

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Now that you have these three distances, represent the location of the genes on the line (chromosome) below:

Optional: This may be done using beads and pipe cleaners. Students need to use rulers to get appropriate distances and provide a key for what gene each color bead represents.



Chestnut leaves with stipules at base of leaf



American Chestnut stems



Male Chestnut flowers

*Traits listed are actual Chestnut traits; however, they are not located on the same chromosome. They are represented in this manner for the purpose of learning how to construct a linkage map.