$\qquad$ Date: $\qquad$ Period: $\qquad$

## How to Make a Linkage Map Using the American Chestnut Tree

Background: 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:

Mating: HhMm x hhmm
Where: $\mathrm{H}=$ Hairy leaves

$$
\begin{aligned}
& \mathrm{h}=\text { Non-hairy leaves } \\
& \mathrm{M}=\text { Male sterile flowers } \\
& \mathrm{m}=\text { Male fertile flowers }
\end{aligned}
$$

Perform the cross:

$$
\begin{array}{|l|l|l|l|}
\hline & & & \\
\hline & & & \\
\hline & & & \\
\hline & & & \\
\hline & & & \\
\hline
\end{array}
$$

How do you tell if your genes are linked? 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 - $850 \quad$ 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 (i.e. crossing over) 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)x 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 trait for hairiness has been placed on the line, measure 15 mm (representative of 15 LMU ) and place the trait for fertility on the stretched out chromosomes. (The line symbolizes a stretched out chromosome):

$$
X^{\text {hairiness }}
$$

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

$$
\begin{array}{ll}
\mathrm{M}=\text { Male sterile flowers } & \mathrm{E}=\text { Early budding } \\
\mathrm{m}=\text { Male fertile flowers } & \mathrm{e}=\text { Late budding }
\end{array}
$$

MmEe is mated to mmee.
Offspring: Sterile/ early- $200 \quad$ Sterile/ late- 600
Fertile/ early- $600 \quad$ Fertile/ late- 200

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. *Note: based on new evidence, you may need to relocate your initial positioning of the fertility gene.
$\mathrm{X}^{\text {hairiness }}$

1. Do the above maps show the exact number of DNA bases in between each gene?
2. Do the maps show how many bases or which types of bases are involved in a particular gene?

Try on your own: Using the following data, build a chromosome map:

| $\mathrm{R}=$ red stems | $\mathrm{L}=$ long male flowers | $\mathrm{W}=$ wide stipules |
| :--- | :--- | :--- |
| $\mathrm{R}=$ green stems | $\mathrm{l}=$ short male flowers | $\mathrm{w}=$ narrow stipules |

RrLl x rrll: 124 Red/ Long LlWw x llww: 1000 Long/ wide RrWw x rrww: 180 Red/ wide

| 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:

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

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

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

