GENIQUEST Guide Part II

Pinpointing Plates

Driving Question-How can the combination of selective breeding, phenotyping, and genotyping help to find the location of a gene?

This section introduces the fundamental concepts of QTL analysis. Dragon plates are revisited and students breed dragons to solve this mystery. Because of genetic recombination, the offspring contain a patchwork of parental genes which is represented in this exercise by colors to track pedigree within chromosomes. Thus the effect of crossing over can be visually explored. Students identify the area of dragon chromosome one most likely to hold the gene(s) for plates by looking for patterns in the genotype shared by plated dragons.

It is important that students understand that this analysis is entirely dependent on the varied patchwork of lineage within each chromosome that occurs as a result of crossing over. . Having a large sample of animals with these patchwork chromosomes is critical to finding out which areas of the genome are related to the disease. By sorting by genotype at intervals along the chromosome(s) and then looking for correlations of these groupings with phenotypes, the most highly correlated intervals can be identified.

Activities to do BEFORE completion of this section:

1. ***Power Point slides and Research Paper can be assigned to students as homework prior to the Professor DePran Section***

Activities following completion of this section:

2. ***Project final graph and discuss***
3. ***Chromosome QTL Puzzle***

Questions to ponder:

1. ***What is the purpose of selective breeding?***
2. ***Which of the two parents appears to be the most inbred? How do you know?***
3. ***What sort of evidence for crossing over did you collect?***
4. ***Why are multiple offspring required?***

Pinpointing Plates- Your big chance has arrived	1

Great news! News of your studies into the location of the plates gene has reached the royal geneticists, and they are very impressed. They have given you a scholarship to study further, and you have been honored with an apprenticeship to the great dragon geneticist Nantal.

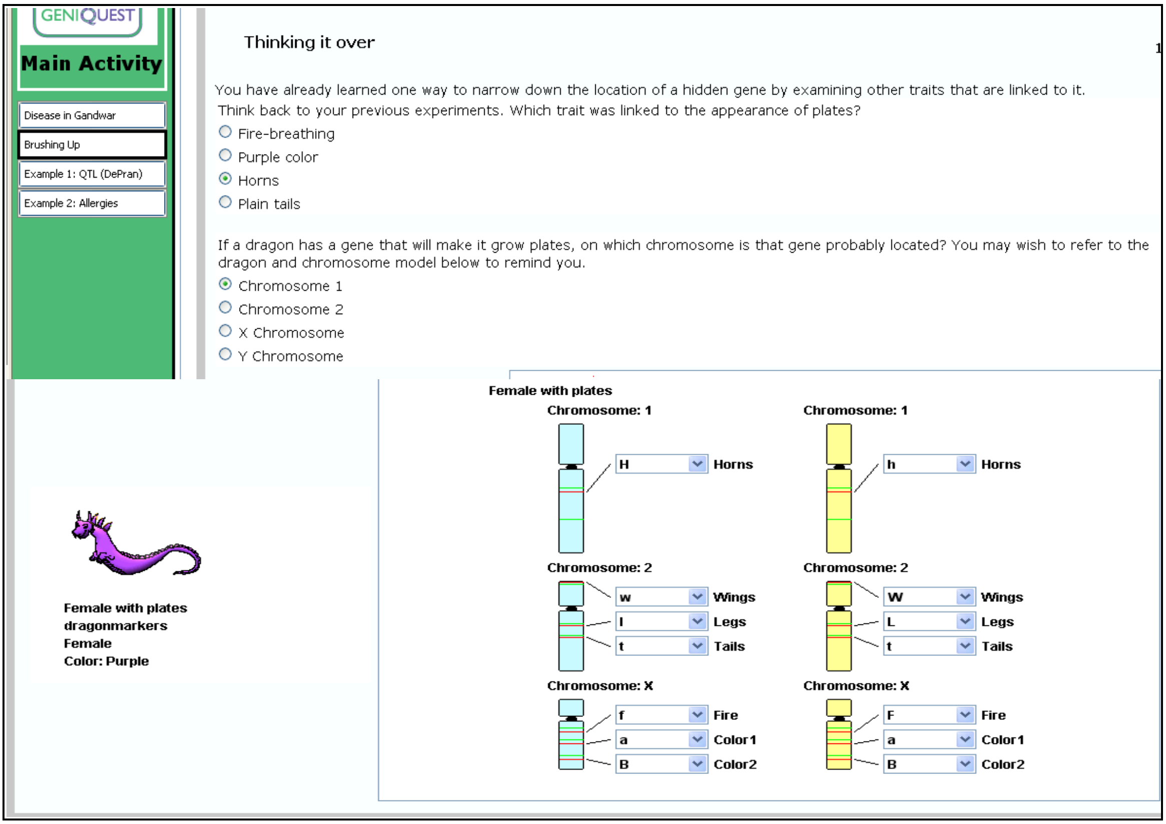
As if that weren't enough, Nantal wants to send you to a scientific conference far across the island to learn more about the latest techniques. Before you can go there, however, the geneticists want you to look further into the interesting question that brought you to their attention. As Nantal's apprentice, you know you will be in great hands. But of course, he wants you to keep thinking about your plates problem right away…

Pinpointing Plates - Thinking it over 2

Thinking it over
You have already learned one way to narrow down the location of a hidden gene by examining other traits that are linked to it.

Think back to your previous experiments. Which trait was linked to the appearance of plates?

If a dragon has a gene that will make it grow plates, on which chromosome is that gene probably located? You may wish to refer to the dragon and chromosome model below to remind you.



Thinking it over

You have already learned one way to narrow down the location of a hidden gene by examining other traits that are linked to it.
Think back to your previous experiments. Which trait was linked to the appearance of plates?

○ Fire-breathing
○ Purple color
● Horns
○ Plain tails

If a dragon has a gene that will make it grow plates, on which chromosome is that gene probably located? You may wish to refer to the dragon and chromosome model below to remind you.

● Chromosome 1
○ Chromosome 2
○ X Chromosome
○ Y Chromosome

Female with plates
dragonmarkers
Female
Color: Purple

Female with plates

Chromosome: 1
H Horns

Chromosome: 2
w Wings
l Legs
t Tails

Chromosome: X
f Fire
a Color1
B Color2

Chromosome: 1
h Horns

Chromosome: 2
W Wings
L Legs
t Tails

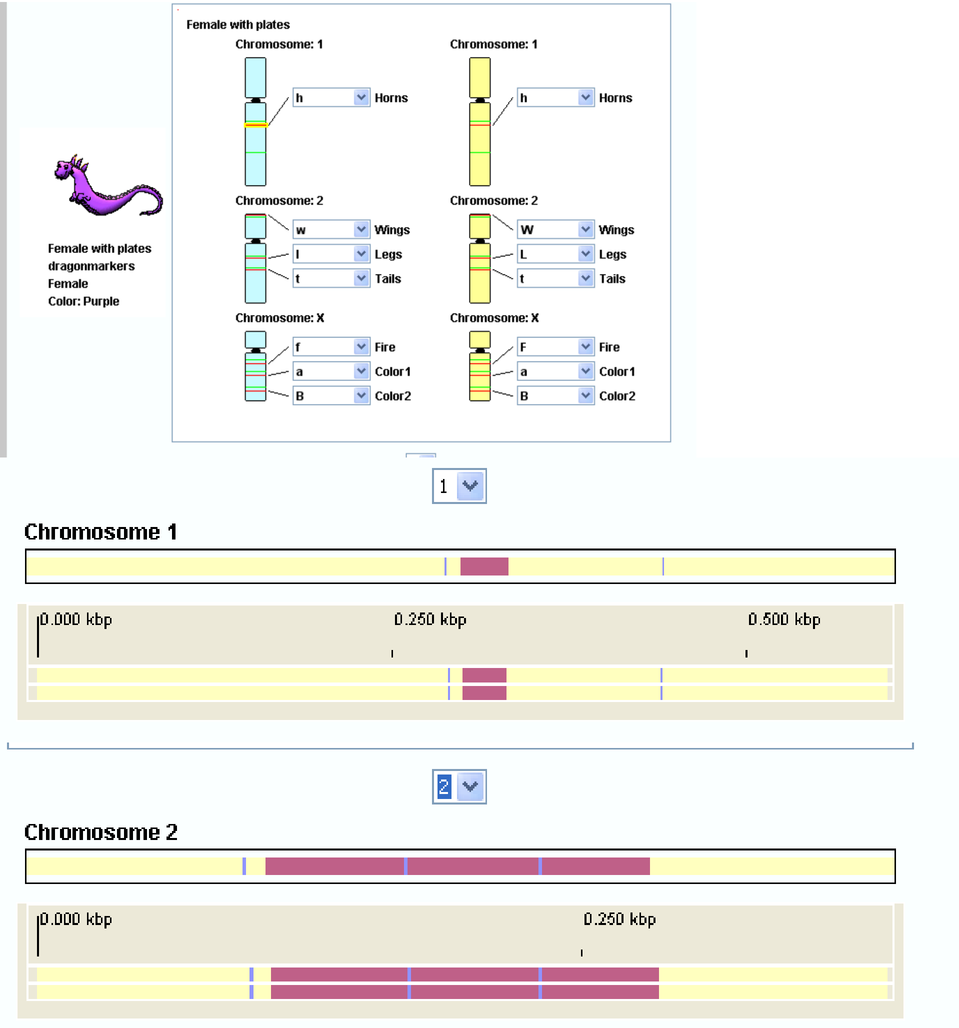
Chromosome: X
F Fire
a Color1
B Color2

Pinpointing Plates - Experimenting

3

Dragons that had plates almost always had horns as well. This makes the trait of horns very useful – if a dragon has horns, it is possible that that dragon may also grow plates. We can say that horns are a genetic marker for the possibility of plates.
Because the gene for horns is on Chromosome 1, we can guess that the hidden gene for plates probably is located there also. We will concentrate on Chromosome 1 in our next experiments (though you can peek at the others if you wish).

Below, you see a dragon and two views of its chromosomes. One of these views is familiar, and the other one is new. Try playing with the lower view and comparing what you see to the upper view. Can you figure out what is being shown here?



Female with plates
dragonmarkers
Female
Color: Purple

Female with plates

Chromosome: 1
Horns: h
Chromosome: 1
Horns: h

Chromosome: 2
Wings: w
Legs: l
Tails: t
Chromosome: 2
Wings: W
Legs: L
Tails: t

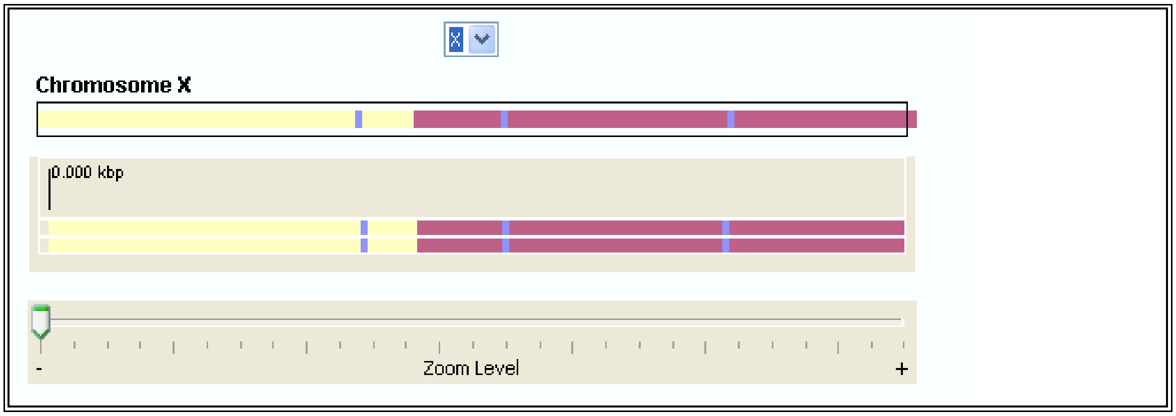
Chromosome: X
Fire: f
Color1: a
Color2: B
Chromosome: X
Fire: F
Color1: a
Color2: B

1

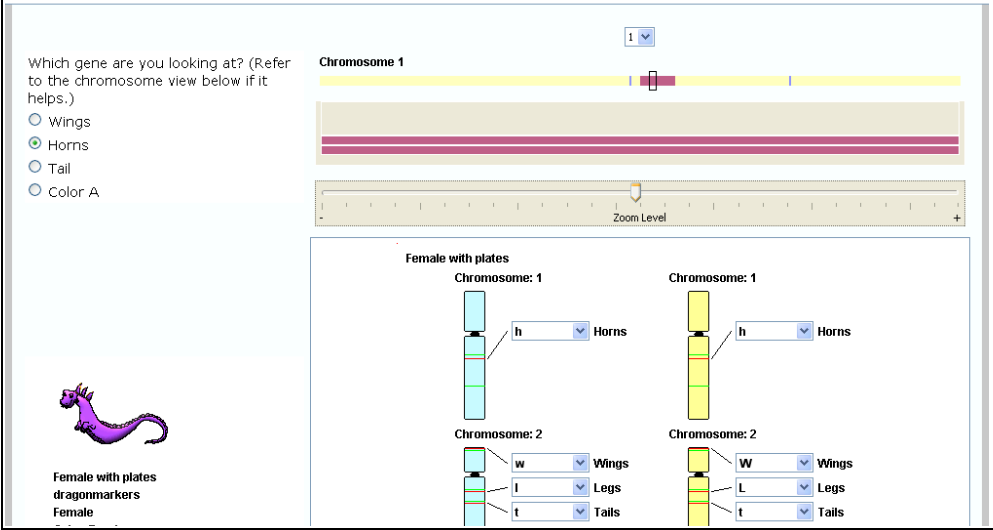
Chromosome 1

2

Chromosome 2



Pinpointing Plates - Zooming in	4
This new view is a zoom-able view of the chromosomes (they're turned on their side compared to the view you're used to). Notice that you see only one set of chromosomes at a time. The black box at the top of the view shows the portion of the chromosome you are seeing at the moment. Try using the Zoom Level slider to zoom in so you are only seeing a small part of the chromosome, and then drag the black box across to the right. Can you fill the view screen entirely with the purple gene? Which gene are you looking at? (Refer to the chromosome view below if it helps.)	



Which gene are you looking at? (Refer to the chromosome view below if it helps.)

○ Wings
● Horns
○ Tail
○ Color A

Female with plates

Chromosome: 1
h Horns

Chromosome: 1
h Horns

Chromosome: 2
w Wings
l Legs
t Tails

Chromosome: 2
W Wings
L Legs
t Tails

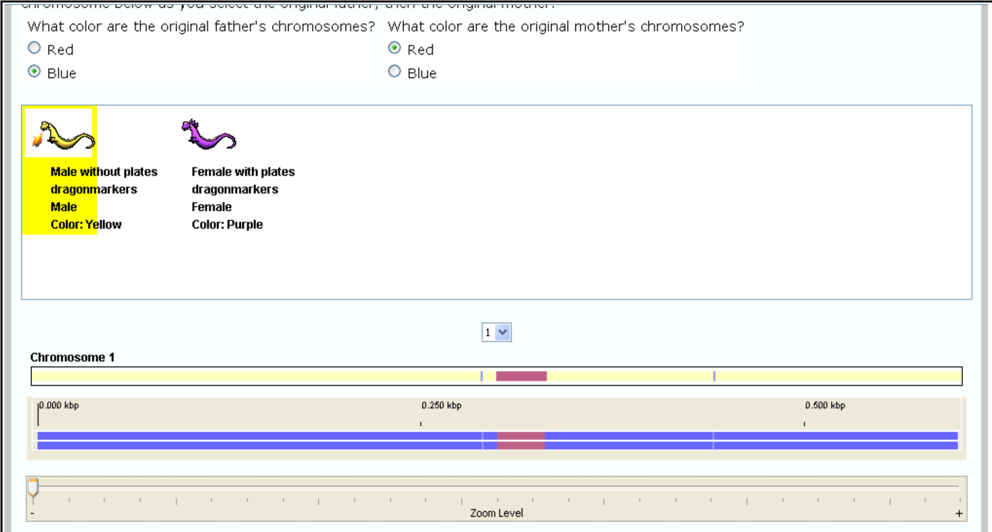
Female with plates
dragonmarkers
Female

Pinpointing Plates- Tracking Chromosomes

5

We'll be breeding two strains together. In doing this, we can help keep track of the organisms we breed by using colors to indicate the organisms of the original pair. Click on each original parent strain organism with your mouse and observe the chromosome shown below.

What color marks the original Strain 1 chromosomes (from the father)?
What color marks the original Strain 1 chromosomes (from the mother)?



What color are the original father's chromosomes?
○ Red
● Blue

What color are the original mother's chromosomes?
● Red
○ Blue

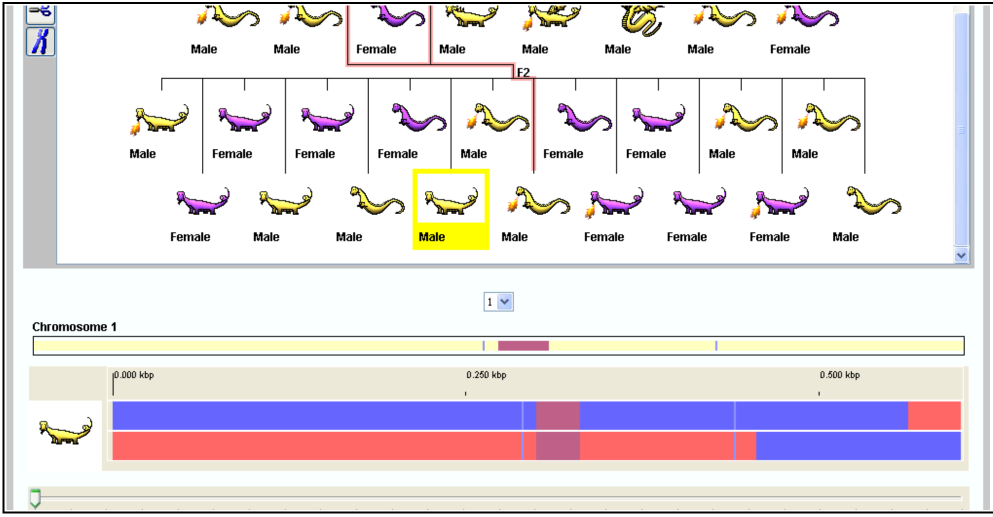
Continue to the next page to breed some dragons.

Pinpointing Plates - Breeding experiments

6

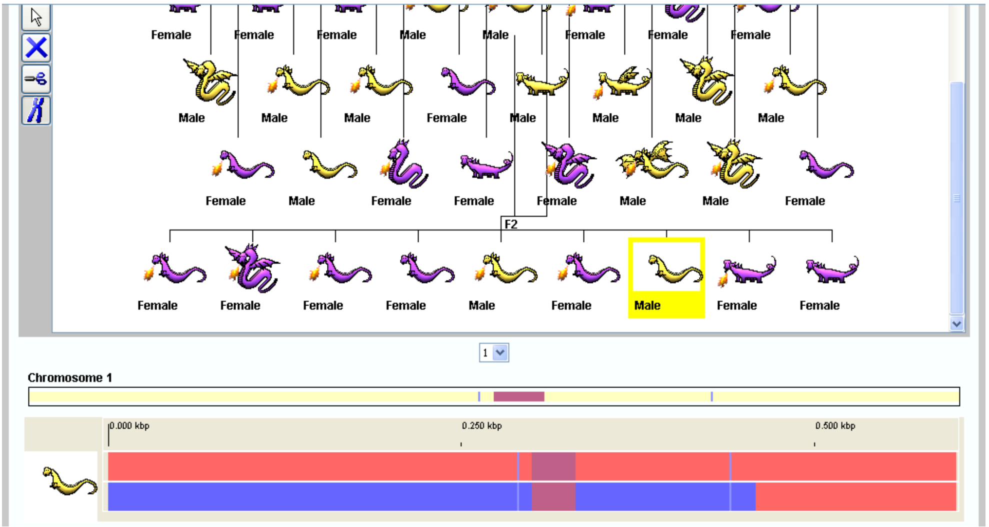
In the space below, you can breed the original dragons as you did earlier, but now you can watch how the first dragons' chromosomes spread through a population. Breed the parents together and examine a few of the dragons

Describe the chromosomes of the dragons in the F2 generation.



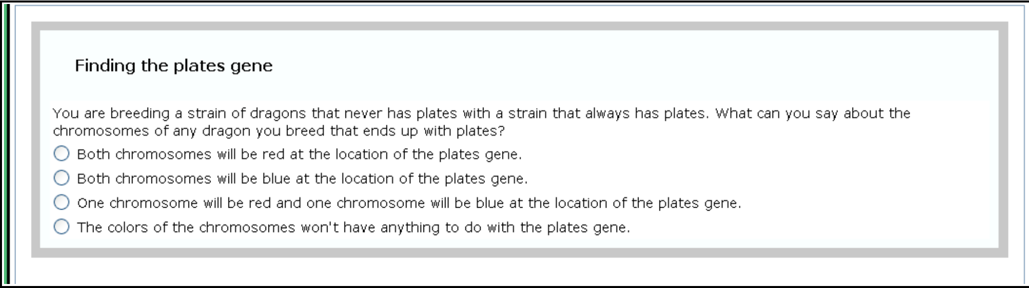
Pinpointing Plates- Making a back cross 7

Now make a *back cross*. Breed the original parents to make an F1 generation. Then take one dragon from the F1 generation and *breed it with the original female*. Examine the individuals in the F2 generation and compare them to the chromosomes of individuals in the F1 generation. (Remember that you can select more than one at a time.)
How are the chromosomes of the F3 generation different from those of the F2 generation? Why?



Pinpointing Plates - Finding the plates gene 8

You are breeding a strain of dragons that never has plates with a strain that always has plates. What can you say about the chromosomes of any dragon you breed that ends up with plates?



Finding the plates gene

You are breeding a strain of dragons that never has plates with a strain that always has plates. What can you say about the chromosomes of any dragon you breed that ends up with plates?

○ Both chromosomes will be red at the location of the plates gene.
○ Both chromosomes will be blue at the location of the plates gene.
○ One chromosome will be red and one chromosome will be blue at the location of the plates gene.
○ The colors of the chromosomes won't have anything to do with the plates gene.

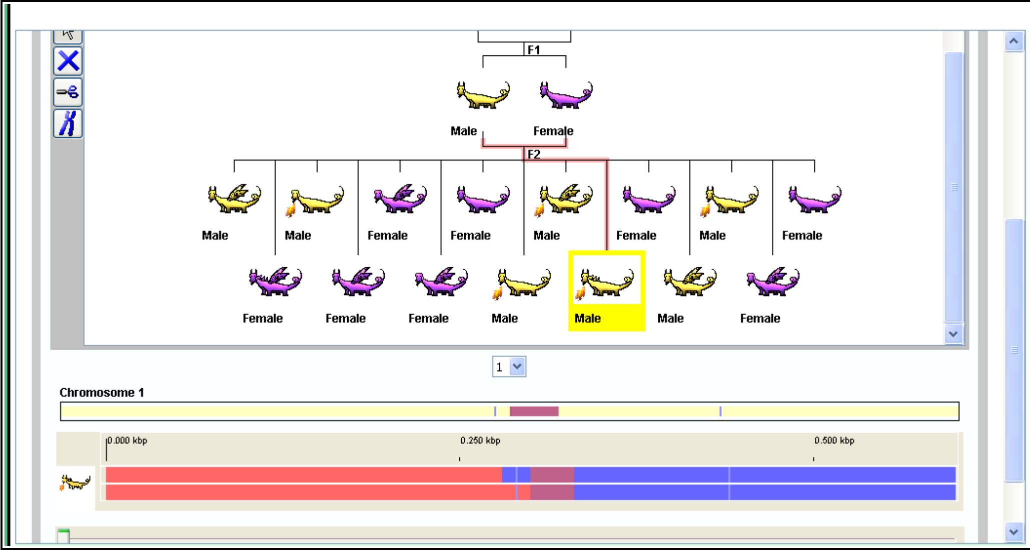
Pinpointing Plates - Finding the plates gene

9

Use this new view of the chromosome to help you search for the gene for plates.

Make another back cross, and use the shift key to select all the dragons that have plates. Do you notice any locations they have in common? It may help to breed several sets of dragons. (The chromosomes are smaller now, to make it easier to see.)

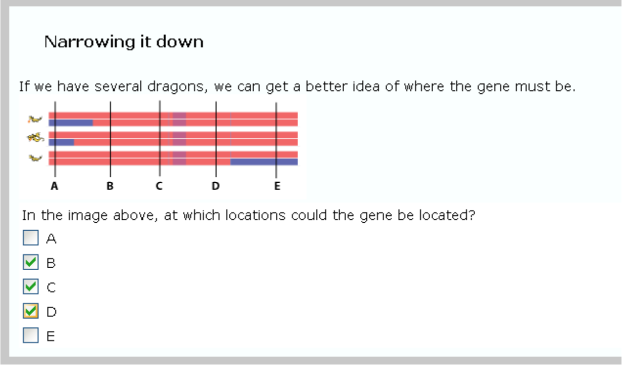
Try selecting some that don't have plates as well. Do these dragons have the same locations in common? Where do you think the plates gene is located?



Pinpointing Plates - Narrowing it down

10

If we have several dragons, we can get a better idea of where the gene must be.



Narrowing it down

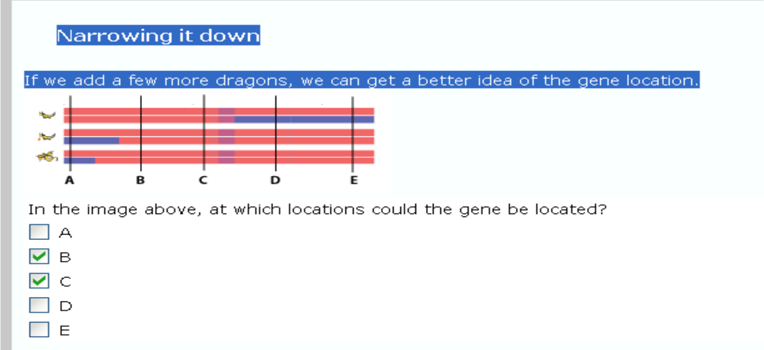
If we have several dragons, we can get a better idea of where the gene must be.

In the image above, at which locations could the gene be located?

☐ A
☑ B
☑ C
☑ D
☐ E

Pinpointing Plates- Narrowing it down	11

If we add a few more dragons, we can get a better idea of the gene location.



Narrowing it down

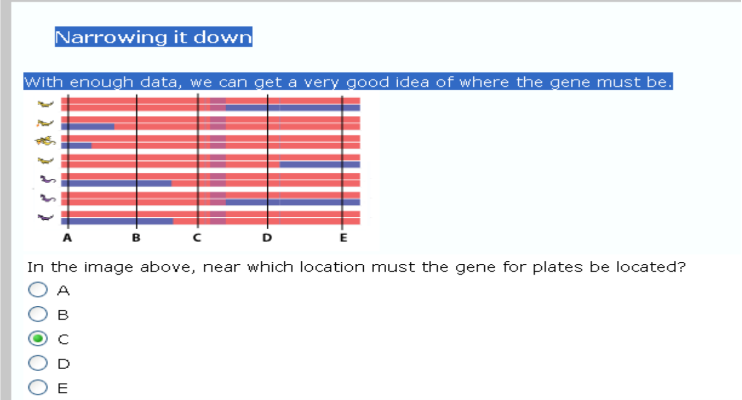
If we add a few more dragons, we can get a better idea of the gene location.

In the image above, at which locations could the gene be located?

☐ A
☑ B
☑ C
☐ D
☐ E

Pinpointing Plates	12

Narrowing it down
With enough data, we can get a very good idea of where the gene must be.



Narrowing it down

With enough data, we can get a very good idea of where the gene must be.

In the image above, near which location must the gene for plates be located?

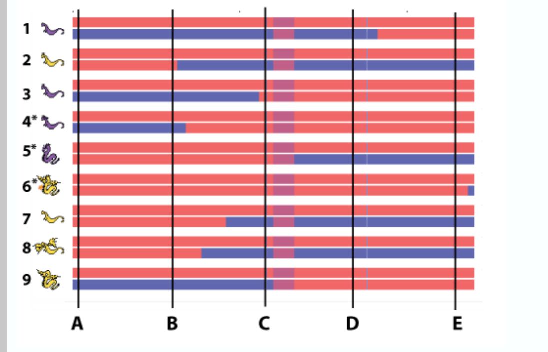
○ A
○ B
● C
○ D
○ E

Pinpointing Plates - Running the numbers

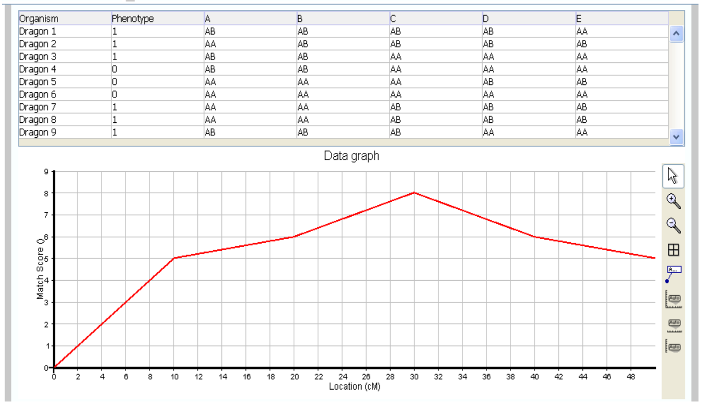
13

So your detective work has told you approximately where the gene for plates is located. Of course, we still don't know exactly where it is, but we know that breeding a lot of dragons (like hundreds) would sure help us narrow it down. It's hard to keep track of so many dragons, though, so it is useful to have another way to show our results.

In the chart below, you can choose two options for the locations A-E. If both a dragon's chromosomes are red at a location, we'll call it *AA*. If one is red and one is blue, we'll call it *AB*. Using the image and the dragons shown, fill out the chart below. *The first three rows have already been completed for you* as an example. Use the image and the dragons shown to **complete the rest of the rows for Dragons 4-9 correctly.** As you complete the chart, pay attention to the graph below.



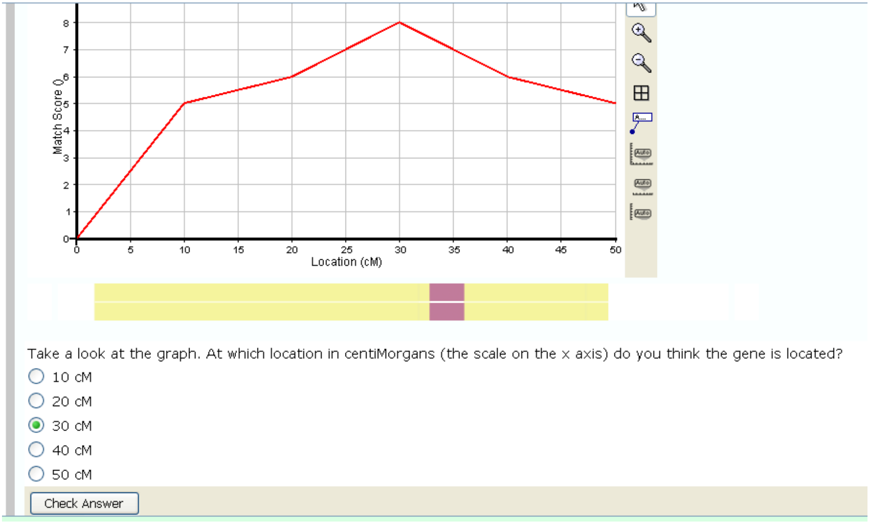
(*Dragons with plates)



Organism	Phenotype	A	B	C	D	E
Dragon 1	1	AB	AB	AB	AB	AA
Dragon 2	1	AA	AB	AB	AB	AB
Dragon 3	1	AB	AB	AA	AA	AA
Dragon 4	0	AB	AB	AA	AA	AA
Dragon 5	0	AA	AA	AA	AB	AB
Dragon 6	0	AA	AA	AA	AA	AA
Dragon 7	1	AA	AA	AB	AB	AB
Dragon 8	1	AA	AA	AB	AB	AB
Dragon 9	1	AB	AB	AB	AA	AA

Pinpointing Plates	14

Running the Numbers
As you filled out the different locations, the graph was keeping score. Every time a dragon without plates was AB, it added a point. Every time a dragon *with* plates was AA, it added a point. When the opposite happened, it took away points. The final score at each location is a kind of "match score" that indicates how likely it is that the gene is found at that location.



Take a look at the graph. At which location in centiMorgans (the scale on the x axis) do you think the gene is located?

○ 10 cM
○ 20 cM
● 30 cM
○ 40 cM
○ 50 cM

Pinpointing Plates - Seeing it in action	14

Great detective work! Now you're finally deemed ready to go to the conference. You can hardly wait, because the legendary scientist VonTenz will be discussing a groundbreaking analysis technique there. You pack your bags and settle into the carriage seat for the treacherous ride over the Tomborg Mountain pass. Fortunately, the seat is comfortable and your driver is experienced, so you feel safe enough to look over an article that Nantar assigned you to read before the conference.

The article is by Dr. DePran, and describes early research into the technique you will hear about at the conference. You pull out the scroll you checked out of the Great Library and begin to read. Journal articles are always so difficult, but you have a feeling that this technique will come in handy sometime…

Professor DePran

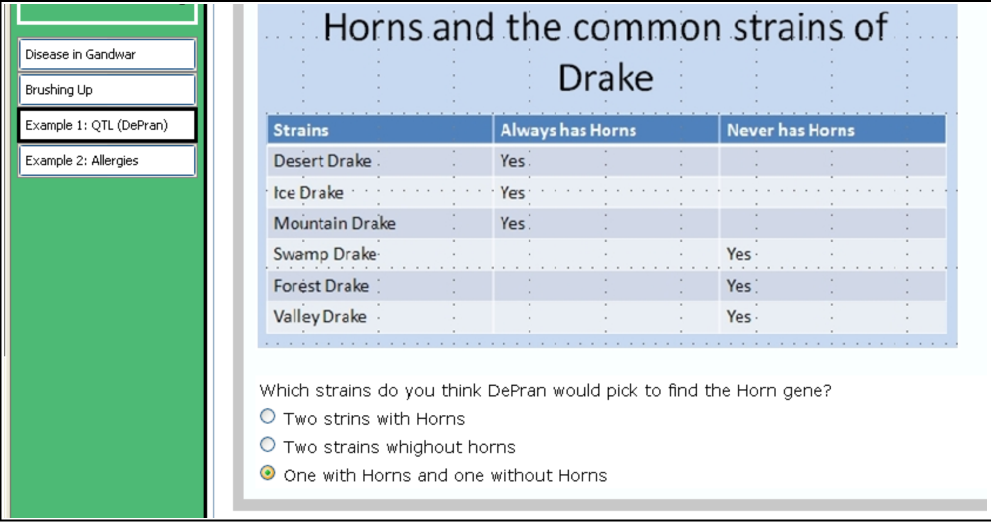
Professor DePran 1

The first talk
The trip to the conference went quickly. Your first conference!
A chance to make friends with other researcher, and the great food!
A chance to listen to talks on the latest techniques, and the great food!
Discussions late into the night about possible new methods, and did we mention, the great food!

The first talk you really wanted to see is just about to start. The famous researcher Professor DePran will be describing his team's discovery of the gene that is responsible for Dragon horns, and the methods he helped develop for using Drakes as a model for Dragon genetics.

Professor DePran: 2

As we embarked on the search for the gene that is responsible for horns in the Dragon, we were confronted by the time it would take to perform our experiments. We need pure lines, inbreed for at least 20 to 40 generations and with the Royal Dragon's slow reproduction rate (once every 500 years) it would take far too long. Fortunately there is a very close model to the Dragon, the Drake. Drakes are small common creatures in the same line as the Dragon but they reproduce 4 times in a year and weigh only an ounce or two. Just as the common mouse has 98% the same genes as a human, the Drakes are 99% the same as the Royal Dragon. Additionally, the Drake lines have been isolated by geography for thousands of years, so the 6 common strains of Drake breed true.



Horns and the common strains of Drake

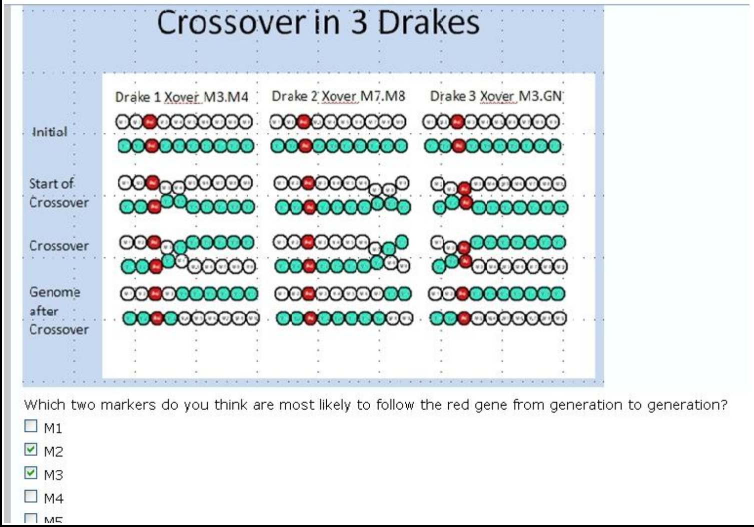
Strains	Always has Horns	Never has Horns
Desert Drake	Yes	
Ice Drake	Yes	
Mountain Drake	Yes	
Swamp Drake		Yes
Forest Drake		Yes
Valley Drake		Yes

Which strains do you think DePran would pick to find the Horn gene?
○ Two strins with Horns
○ Two strains whighout horns
● One with Horns and one without Horns

Professor DePran:	3

We picked one strain with horns and one strain without horns to breed and test. We have recently sequenced the genome of all the common strains of Drake but it would be far too expensive to do this for all of our test animals. Instead we used “markers”, sometimes called SNPs for Single Nucleotide Polymorphisms. SNPs are a change in a single base pair that are different between two strains of Drake. By testing for these we can deduce which strain the region around the SNPs came from. Here is a simple example for three drakes. The red dot is a chromosome that we normally cannot see. Each generation there is recombination or “crossover”. I have colored the markers of one strain in white and the other in blue. If this crossing over occurs once for each generation and the distribution along the genome is random then we would expect the markers closest to the gene of interest to more often accompany the trait. And this is true.

Which two markers do you think are most likely to follow the red gene from generation to generation?



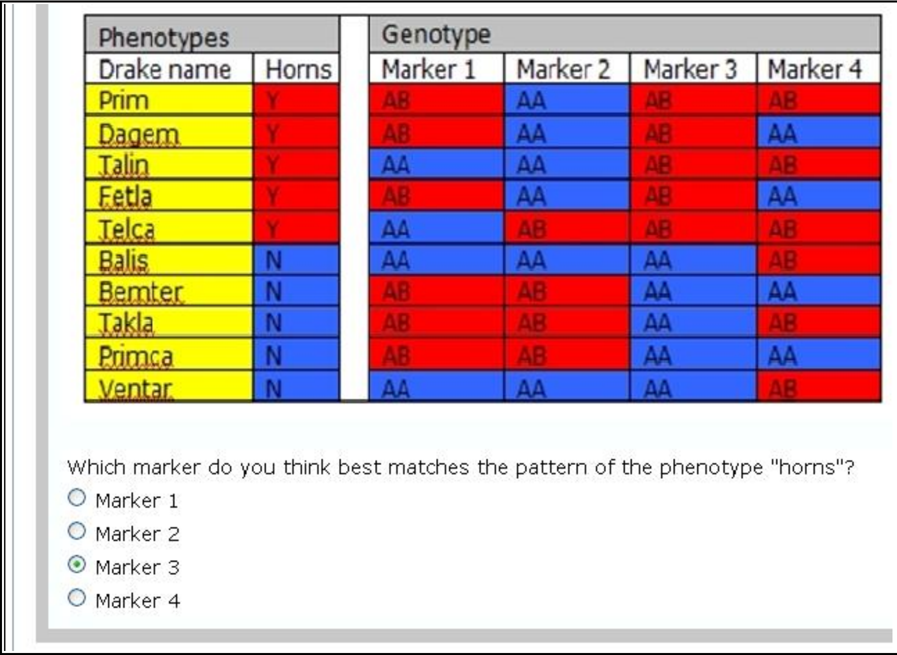
Which two markers do you think are most likely to follow the red gene from generation to generation?

☐ M1
☑ M2
☑ M3
☐ M4
☐ M5

Professor DePran:	4

The frequency of recombination between two locations is a measure of the distance between two alleles. We can use this to find the markers that are closest to the genes of interest by observing the phenotype (horns) and genotyping the markers. Here is an example in tabular form:

Which marker do you think best matches the pattern of the phenotype "horns"?



Phenotypes		Genotype			
Drake name	Horns	Marker 1	Marker 2	Marker 3	Marker 4
Prim	Y	AB	AA	AB	AB
Dagem	Y	AB	AA	AB	AA
Talin	Y	AA	AA	AB	AB
Fetla	Y	AB	AA	AB	AA
Telca	Y	AA	AB	AB	AB
Balis	N	AA	AA	AA	AB
Bemter	N	AB	AB	AA	AA
Takla	N	AB	AB	AA	AB
Primca	N	AB	AB	AA	AA
Ventar	N	AA	AA	AA	AB

Which marker do you think best matches the pattern of the phenotype "horns"?

- ○ Marker 1
- ○ Marker 2
- ● Marker 3
- ○ Marker 4

Professor DePran:	5

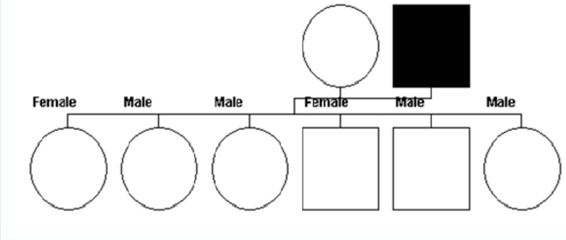
Looking at the patterns we can see that Marker 3 is a perfect match for the pattern we see in the horn phenotype. We can simply count up the matches and graph them to more easily see the results:

Phenotypes			Genotype			
Drake name	Horns		Marker 1	Marker 2	Marker 3	Marker 4
Prim	Y		AB	AA	AB	AB
Dagem	Y		AB	AA	AB	AA
Talin	Y		AA	AA	AB	AB
Fetla	Y		AB	AA	AB	AA
Telca	Y		AA	AB	AB	AB
Balis	N		AA	AA	AA	AB
Bemter	N		AB	AB	AA	AA
Takla	N		AB	AB	AA	AB
Primca	N		AB	AB	AA	AA
Ventar	N		AA	AA	AA	AB

Of course when you have hundreds of Drakes and hundreds of markers it is much easier to have a statistical test that gives a number for each marker. We call this a LOD score. LOD is the Log (base 10) of the Odds of finding the gene at a particular location. The higher the LOD score the more likely we are to find the Gene in that location. This also gives us the ability to quantify thresholds and confidence intervals. Thresholds tell us how high a LOD score we need for a given probability. For example, we may want a threshold line that represents a 1 in 100 probability that we would see a LOD score that high by pure chance. Confidence intervals allow us to determine the extent of the region on the chromosome. For example, we can specify that the gene in question has a 95% chance of being between 400,000 and 450,000 base pairs on chromosome 1.

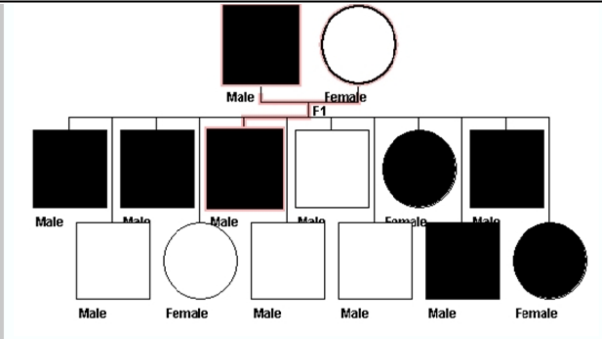
Professor DePran: 6

We created a cross between Desert Drakes and Valley Drakes. Desert Drakes always have horns and Valley Drakes never have horns. After the first cross ALL of the Drakes had horns.



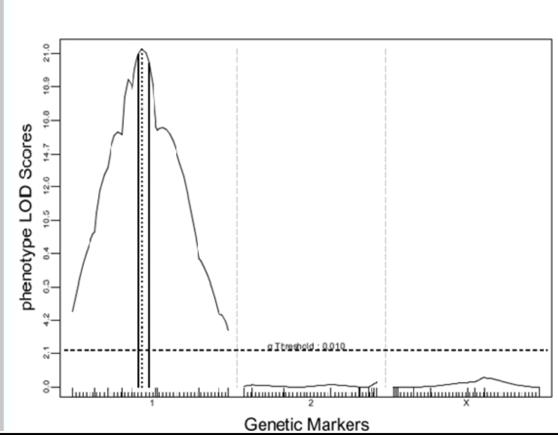
White is for drakes with horns, black is for drakes without horns. Round is Female, square is male. The result of ALL drakes in the first generation having horns confirmed that horns is a dominant trait. If “H” is the allele for horns and “h” is the allele for no horns then: HH = horns Hh = horns hh = no horns.

For the Second generation we will cross a hh Male with a Hh Female:



Professor DePran:	7

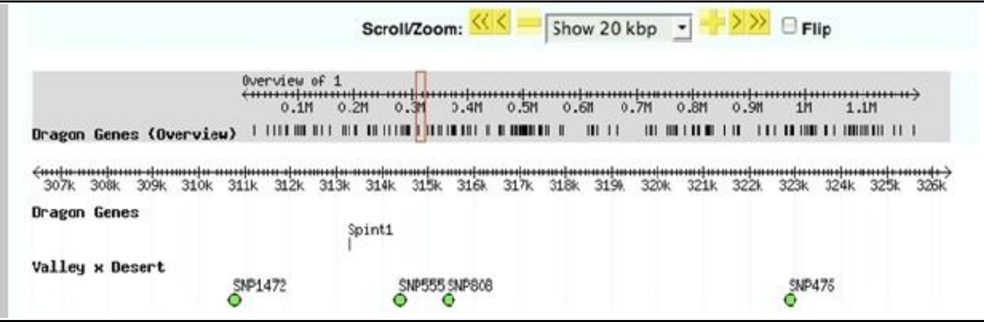
For our QTL (Quantitative Trait Loci) analysis we took Males from this generation of Heterozygote (mixed) Drakes that had horns and crossed them to Valley drakes that did not have horns (as seen in the second figure on the previous slide).
We created 418 Drakes for this cross and measured their phenotype. We genotyped the drakes at 200 markers and generated the LOD scores.



The threshold line shows that the LOD score is much higher than 1 chance in 100. The 95% Confidence Interval bars are from 310,000 to 320,000 base pairs on chromosome 1.

Professor DePran:	8

Over years of research we have accumulated vast knowledge about different segments of the genome. Archivists and curators have gathered this information and collected it in large data bases. One of the ways we index this information is in the order it occurs in the genome. These are the Genome Browsers. With the recent addition of the full sequence on many organisms (including human, mouse, dragon, and drake) we now have a way of looking at what features occur in the segment between 310,000 and 320,000 base pairs on chromosome 1.
When we go to the genome browser and look in this region we find only one gene!



And that one gene is Spint1 .
Knockouts (deactivation) of the Spint1 gene confirmed that this did prevent the formation of horns in the Desert Drake that would normally have horns.

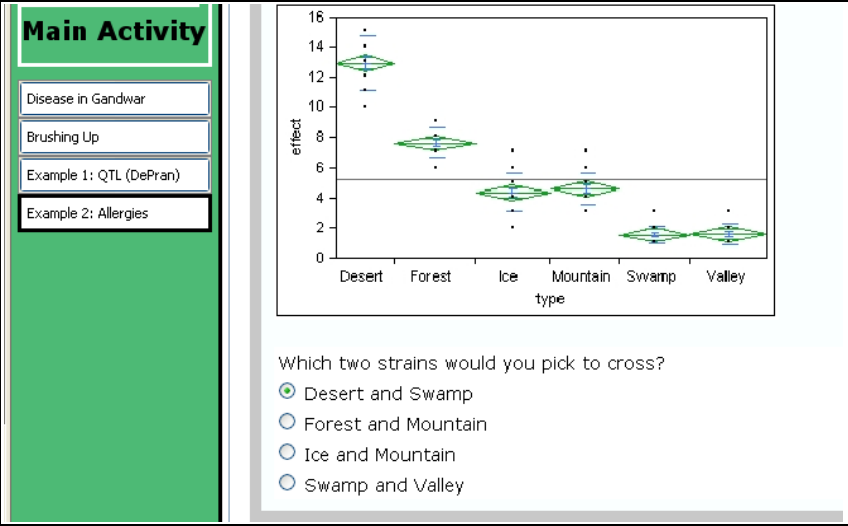
Thank you for your time, I will now take questions……

Professor DePran	8
That was a very informative talk and covered many of the techniques that will be needed to hunt down the cause of ScaleBlanche. So much information, but there is no time to do anything but jot down some quick notes and get ready for the next talk…. You have been looking forward to meeting Professor vonTenz. She was DePran’s best student and has continued his work, extending it to the search for the cause of disease. Now we will get to see how these methods can be applied by one of the best...	

Professor vonTenz

Professor vonTenz: 1

Professor vonTenz
The following graph shows the severity of the effect (as measured by the pigment change in the face two hours after ingestion of a diet containing 22% Allium cepa {in the form of Salsa, extra hot}: (diamonds show the Mean value and the bars show two standard deviations).



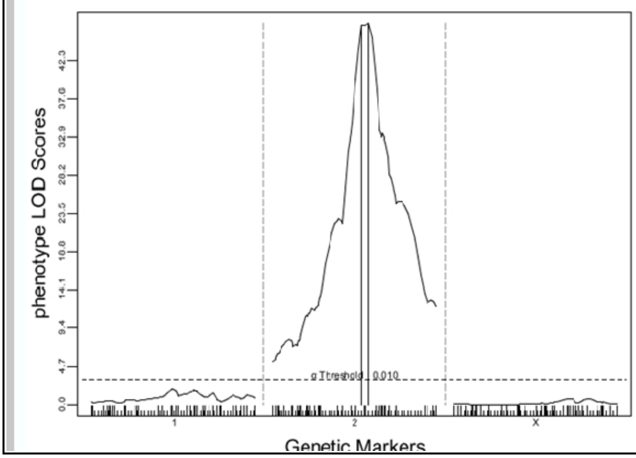
Which two strains would you pick to cross?

● Desert and Swamp
○ Forest and Mountain
○ Ice and Mountain
○ Swamp and Valley

Professor vonTenz: 2

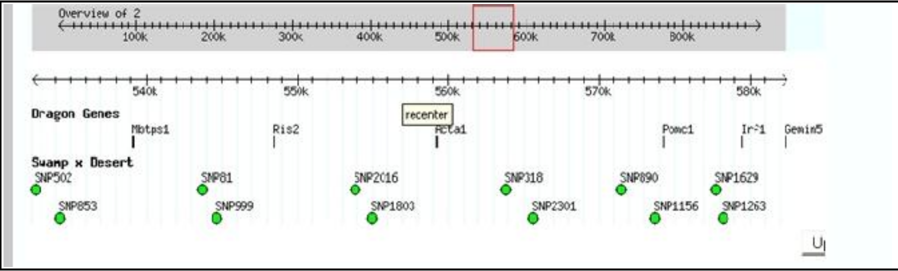
We determined that the allergy was a recessive trait and that the Swamp drake was homozygous for resistance (RR) and the Desert Drake was homozygous for the Allergy (rr). We choose to cross the Swamp drake with the Desert Drake to generate heterozygous offspring (Rr). We then crossed these offspring to generate 506 second generation offspring (RR. Rr. rr). These were tested for quantitative face pigment change and genotyped.

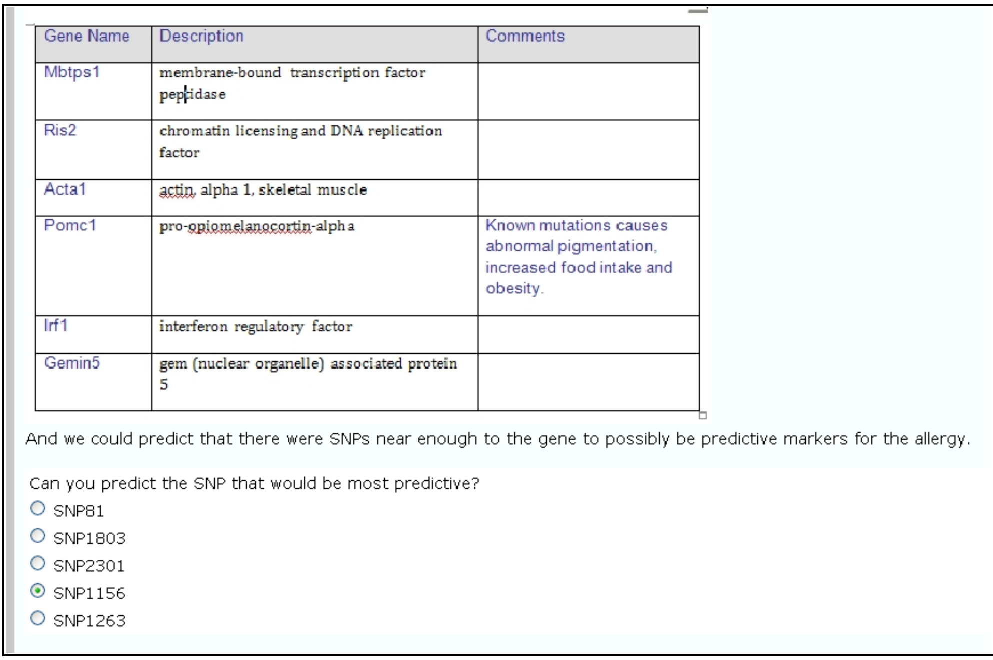
What percent of the offspring would you expect to show a strong allergy? 25%

<table>
<tr><th>Professor vonTenz:</th><th>3</th></tr>
<tr><td colspan="2">We expect 25% of the offspring to be RR, 50% to be Rh (for the Rh and hR, we didn’t measure if the “R” came from dad or mom) and 25% rr. We observed a strong allergy effect in about 25% of our second generation drakes. Our QTL analysis used 30 markers on each chromosome and showed a strong peak on Chromosome 2. The large LOD score is indicative of a single gene. The Confidence Interval gave a 95% chance that the gene was between 54 and 58 centiMorgans (540,000 and 580,000 bp).</td></tr>
<tr><td colspan="2">
</td></tr>
</table>

Professor vonTenz: 4

Going to our Genome Browser and looking at this region of Chromosome 2 gives us five possible genes:





Gene Name	Description	Comments
Mbtps1	membrane-bound transcription factor peptidase	
Ris2	chromatin licensing and DNA replication factor	
Acta1	actin, alpha 1, skeletal muscle	
Pomc1	pro-opiomelanocortin-alpha	Known mutations causes abnormal pigmentation, increased food intake and obesity.
Irf1	interferon regulatory factor	
Gemin5	gem (nuclear organelle) associated protein 5	

And we could predict that there were SNPs near enough to the gene to possibly be predictive markers for the allergy.

Can you predict the SNP that would be most predictive?

- ○ SNP81
- ○ SNP1803
- ○ SNP2301
- ● SNP1156
- ○ SNP1263

Professor vonTenz: 5

We found the most predictive SNP to be SNP1156, giving an almost 99% prediction of the alleles and predicting the allergic effect. Through this work we will now be able to predict from a simple and inexpensive test if a Dragon will be allergic to Allium cepa and freeing our great friends to enjoy their Salsa without fear. Additional work may lead to a way of correcting the mutation in the Pomc1 gene in the future.

Thank you for your attention, I will now answer questions…….

Interlude	1
We had no idea of the massive amount of information that is available in the archives. Thousands of researchers working for decades to create and curate knowledge, all now at our fingertips. So much and yet it covers less than 3% of the known genome. But the power of the techniques gives us hope that our search is now possible. We can go over our notes on the trip back to home, and start to make research plans…. Now go to the next section and you can start your own research to find the gene that causes ScaleBlanche!	