MIDTERM1 REVIEW QUESTIONS :

1. Several mutants of grasshoppers were found: the first type, a lazy jumper that jumped less frequently and not very far compared to wild-type grasshoppers and the second type a grasshopper that did not chirp. You want to determine something about the inheritance of these mutations.

a. Why do we cross each of these mutants to true-breeding wild-type first?

Determine if true breeding, determine dominance relationship to wild-type allele.

When the lazy jumper was crossed to a true-breeding wild-type grasshopper: 79 wild-type and 81 lazy jumpers were produced. When two lazy jumpers were crossed to each other 80 lazy jumpers and 40 wild-type and were produced. After 70 crosses between lazy jumpers a similar ratio of wild-type to lazy jumpers were always produced.

b. Create a hypothesis to account for this result.

lazy jumpers are heterozygous

J- = lazy J+ = wildtype J- > J+

J-/J- are lethal

LZ = lazy LZ+ = normal jumping

LZ > LZ+

LZ/LZ+ x LZ+/LZ+

→ F1 ½ LZ/LZ+ lazy

½ LZ+/LZ+ WT

✓ fits data from 1st cross

When the non-chirping grasshopper was crossed to true-breeding wild-type the F1 were all wild-type but the F2 produced 119 wild-type chirping grasshoppers and 40 non-chirping grasshoppers

c. What do you conclude about non-chirping?

F1 all wildtype... non chirping is recessive to wildtype and heterozygous

3:1 expected for WT > non chirping

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d. If you cross a number of non-chirping females to a number of lazy jumper males what do you expect to see in the F1 and F2?

Because the previous information didn't say anything about sex differences in the F1 or F2 the mutations must be autosomal.
non-chirping is C- and chirping is C+; C+ > C- (based on part d)
I assume that non-chirping females are homozygous for wild-type jumping and that lazy jumper males are homozygous for wildtype chirping.
I also assume that the two mutations will assort independently
C-/C- ; J+/J+ x C+/C+ ; J+/J-
F1: ½ C+/C- ; J+/J+ (chirping, normal jumpers) : ½ C+/C- ; J+/J- (chirping, lazy jumpers)
F2: I will cross the dihybrid (chirping het, lazy jumpers) to each other
C+/C-; J+/J- x C+/C- ; J+/J-
¾ C+/_ x ½ J+/J- = 6/16th normal chirping, lazy jumping → 6/12th
¾ C+/_ x ¼ J+/J+ =3/16th normal chirping, normal jumping → 3/12th
¾ C+/C_ x ¼ J-/J- = 3/16th dead
¼ C-/C- x ½ J+/J- = 2/16th non-chirping, lazy jumpers → 2/12th
¼ C-/C- x ¼ J+/J+ = 1/16th non chirping normal jumping → 1/12th
¼ C-/C- x ¼ J-/J- = 1/16th dead
RENORMALIZE to remove 4/16th dead

2. Loppins (*Loppinicus loopy*) are fictitious, but very useful diploid invertebrates with a total of 6 chromosomes in their somatic cells. Of those 6 chromosomes, 4 are autosomes and 2 are sex chromosomes. Like humans, male loppins are XY while females are XX.
The gene that determines loppins' blood type is called *bt* and is on chromosome 1, the gene that determines the presence or absence of eyelashes is called *eye* and is on chromosome 2, and the gene that determines ability to digest cellulose is called *cel* and is on the X chromosome.
bt^A, bt^B; $eye^{W,}$ eye^D, *celWT* and *celM* are alleles of these three genes.

a) A female loppin is a triple heterozygous with the genotype bt^A/bt^B; eye^{WT}/eye^D; X^{celWT}/X^{celM}. Her mother was homozygous for bt^A, for eye^D and for *celWT*.
Draw a somatic cell of our triple heterozygous female loppin in G1 stage of the cell cycle (that is, before DNA replication). Make sure that the chromosomes are properly drawn and clearly label all the relevant genes and alleles.
-pay attention to whether the students have created 3 chromosomes that are distinctly different from each other

b) What alleles did the triple heterozygous female loppin inherit from her mother, and what alleles did she inherit from her father?
-this is practice with the problem-solving skills covered in lecture. And you can tell them my rule which is the "Its gotta come from somewhere" rule. They were told the genotype of the mother so that allows them to determine the alleles from the mother. What's left has to come from the father.

c) For research purposes you remove three meiocytes from the triple heterozygous female, you let them undergo the cell cycle and meiosis, and you analyze the genotype of the gametes that are produced. The first meiocyte produces two gametes of genotype bt^A; eye^{WT}; X^{celWT} and two gametes of genotype bt^B; eye^D; X^{celM}.

i) Draw this meiocyte at metaphase of meiosis I (this is when the homologous chromosomes are paired and lined up in the centre of the cell). Make sure to clearly label all the relevant genes and alleles.

-remind the students that how the chromosomes line up at metaphase I is what sets up the whole pattern of segregation for creating the gametes.

ii) The second meiocyte produces two gametes of genotype bt^B; eye^{WT}; X^{celWT} and two gametes of genotype bt^A; eye^D; X^{celM}. Explain what must have happened differently in this meiocyte compared to the one in part i) to produce this result. You may refer to the diagram that you drew above to illustrate your rationale.
-Independent Assortment in action! You could suggest they draw this meiocyte in metaphase I also to visualize the difference. Remember Dr. Klenz says always draw your chromosomes.

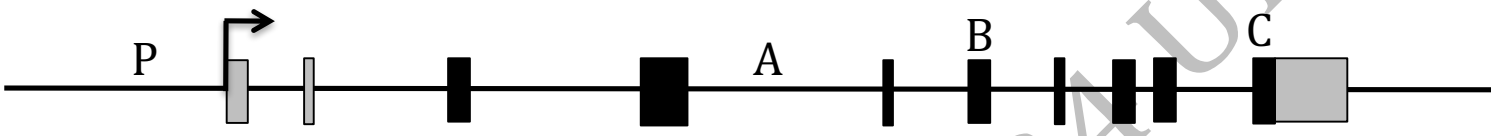
iii) The third meiocyte produces one gamete of genotype bt^A; eye^{WT}; X^{celWT}, one gamete of genotype bt^B; eye^{WT}; X^{celWT}, one gamete of genotype bt^B; eye^D; X^{celM} and one gamete of genotype bt^A; eye^D; X^{celM}. Briefly explain what must have happened to produce this result. You may refer to previous drawings if you need to.
-we haven't discussed crossing over yet but they have had it in previous classes. If they remember that the lineup in metaphase I sets the pattern for gamete segregation then how else can you explain it

iv) If we looked at the gametes produced by 100 different meiocytes from this triple heterozygous female loppin, what are all the genotypes that we would expect to find, and in what proportions? *(Note: 100 different meiocytes will produce a total of 400 gametes)*

As each gene is on a different chromosome then everything is assorting independently so each type of gamete is equally likely. They should be able to come up with all the combinations without too much difficulty.

3. Below is shown the gene structure of the gene *isocitrate dehydrogenase* (*ic*) from yeast *Saccharomyces cerevisiae* . Based on sequence analysis there are no known enhancers in this gene. The protein coded for by this gene is an enzyme within the citric acid cycle operating in the mitochondria that converts isocitrate to alpha ketoglutarate. The ultimate purpose of this cycle is produce reduced NADH which is then converted to energy through other major metabolic reactions. Yeast cells that cannot produce NADH through this cycle then have to perform anerobic fermentation to obtain energy.

The locations of 3 different mutations are shown.



Mutation A is an insertion of 2 nucleotides in the middle of the 4th intron.
Mutation B is the deletion of 4 nucleotides in the 6th exon, which also happens to be part of the active site of the enzyme.
Mutation C is the substitution of 1 nucleotide which causes a missense mutation in the last exon and causes the enzyme to be found in the cytoplasm rather than the mitochondria where it is normally found.

a) In the table below explain what the phenotype of each of these mutations will likely be (when homozygous) at the different levels:

genotype	mRNA (sequence, size amount)	Protein (sequence, size, amount, function)	Whole organism (single yeast cell)
ic^A/ic^A	mRNA amount is normal in size and amount	Wt sequence, size, amount and function (assuming no enhancers in 4th intron, assume splicing not disrupted)	Cell has normal metabolism
ic^B/ic^B	mRNA amount is normal but 4 nucleotides shorter so very slightly lower on gel	Sequence is wt up to the point of the mutation when there is then a frameshift which changes the sequence and also likely makes the protein shorter. This protein is non-functional because the active site is destroyed	This cell cannot perform aerobic respiration and must ferment to obtain energy. Cell is likely smaller and divides more slowly.
ic^C/ic^C	mRNA is normal size and amount	Protein sequence is the same except for one amino acid near the end. It is likely functional or has very slightly reduced function	Because the protein is not found where it needs to function, this cell cannot perform aerobic respiration and must ferment to obtain energy. Cell is likely smaller and divides more slowly.

b) If the normal job of isocitrate dehydrogenase is to convert isocitrate into alpha ketoglutarate explain whether or not each mutation could be rescued by adding alpha ketoglutarate to the cell (and we are assuming the alpha ketoglutarate makes it into the mitochondria)
Mutation A already has wildtype phenotype so adding alpha ketoglutarate is not needed.
Mutation B and C fail to produce functional protein in the mitochondrial so they both could both be rescued by adding alpha ketoglutarate.

c) Rank and explain the relative severity of the three heterozygous allele combinations. State any assumptions you are making.

ic^A/ic^B; assuming ic^A is haplosufficient this heterozygote will have wildtype phenotype

ic^A/ic^C; assuming ic^A is haplosufficient this heterozygote will have wildtype phenotype (it should be equivalent to ic^A/ic^B

ic^B/ic^C as neither allele makes a functional protein this combination will basically be a null and will be the most severe

4. Sex linkage in dogs is similar to humans. An unusual female bulldog is found that walks on its tiptoes. When this bulldog is crossed to a 'wildtype' (not tiptoeing) bulldog, all their offspring tiptoe.

How could we evaluate whether there is an environmental vs. genetic cause of tiptoeing?

-Let the brother and sister pups mate and observe segregation of the tiptoeing phenotype in the F2.
-Raise another litter of pups from these parents in a separate environment away from their mother and observe that they still tiptoe.
-Raise some unrelated bulldog pups in this environment and observe that they don't tiptoe

What could be an environmental cause of the tiptoeing phenotype we observe in the female bulldog and all her pups? Speculate

Eg. –The owner cuts off the hind most claw (the nail) on all her pups feet. This is painful and causes the young dogs to walk on tiptoe and it is a habit they never unlearn.
- The female bulldog and her pups like to watch ballet dancers on television

If all environmental causes of the phenotype are ruled out then what can we conclude about the inheritance of the tiptoeing mutation from the information above

The mutation is dominant

What would allow you to tell if the mutation is autosomal or X-linked? (Suggest two things)

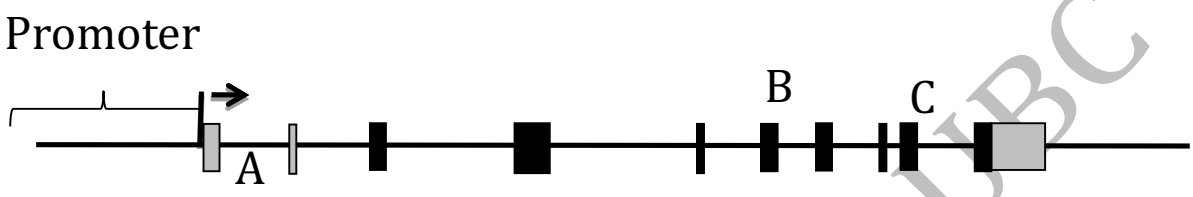
If did the reciprocal cross (tiptoeing male x normal female) the result of the F1 would tell you
If you looked at the F2 from the above cross (crossed F1 tiptoeing brother and sisters)

Suggestions to help students : *Try writing out the genotypes of this first cross assuming it is X-linked with the proper notation. Write out the genotypes of the reciprocal cross assuming sex linkage. What phenotypes are seen in the F1 of the reciprocal cross. Next write out both crosses assuming the mutation is autosomal. What are the phenotypes in the F1?*

5. Researchers are investigating a rare condition in humans called congenital insensitivity to pain, or CIP. The condition results in the inability to sense pain (e.g. if cut, a person or mouse with CIP can be unaware they cut themselves and will not experience pain). If they discover more about this condition, it could provide useful information for the development of medicine to treat pain disorders. You also have a second population of people that have the condition: extreme pain disorder (EPD). People with EPD can experience feelings of pain even when there is no painful stimulus.

From your research you have determined that both CIP and EPD are the result of mutations in the *same* gene, called *SCN9A*. *SCN9A* codes for a sodium channel protein that allows action potentials to occur when stimulated. When stimulated, the channels open and sodium ions flood into the cell, causing an action potential and the ability to feel pain.

The locations of 3 different known mutations in SCN9A are shown below.



Mutation A is an insertion of 25 nucleotides in the middle of the 1st intron.
Mutation B is a base substitution in the 6th exon.
Mutation C is a base substitution in the 3' splice site of the 9th intron.

a. For each mutation, predict if you think it is involved in either of the disorders, and if so, which one (CIP or EPD). Explain your prediction and state any assumptions you are making and/or if more information is needed to make a more accurate prediction.

A – could make an enhancer non-functional, resulting in reduced expression causing CIP. OR, it could make the enhancer more functional (e.g. recruiting more transcription factors) thus causing EPD. However, a 25 bp insertion causing an enhancer to be more functional seems less likely, so I would predict that mutation A is most likely associated with CIP.
B – Base substitution could result in the protein being non-functional if it's a conserved or very important amino acid. This could cause CIP. Also, if the base substitution causes the channel to open when it shouldn't, this could cause EPD. So, I predict this mutation could be involved in either CIP or EPD. We would need more info on the change, and how it affects the protein function.
C - If splice site removed because of base substitution, the last intron could be included in the mRNA. This would likely cause a significant change in 3' end of the protein sequence (additional bases, perhaps an early stop or a frameshift) which would likely cause the protein to not function, causing CIP or EPD.

b. What do you predict the phenotype would be for a person with the following genotypes? Explain. Note that $SCN9A^{+}$ is the wild-type allele, and the superscript A, B, C refer to the alleles mentioned in part a.

Note, your answers might vary slightly if you made different predictions in a, or if you made different assumptions about the haplosufficiency of the alleles.

Assumptions I am making: CIP is the result of a loss-of-function mutation (LOF) , EPD is the result of a gain-of-function mutation (GOF). In the case of an LOF I would predict the wild-type version is haplosufficient. In the case of a GOF I would assume the wild-type is recessive, and a single copy of a GOF mutation is sufficient to cause EPD. Also, remembering what I predicted above: A – CIP, B – EPD or CIP (but for this answer I'll commit to one prediction, EPD), C – CIP.

i. $SCN9A^{+}/SCN9A^{A}$
ii. $SCN9A^{+}/SCN9A^{B}$
iii. $SCN9A^{+}/SCN9A^{C}$

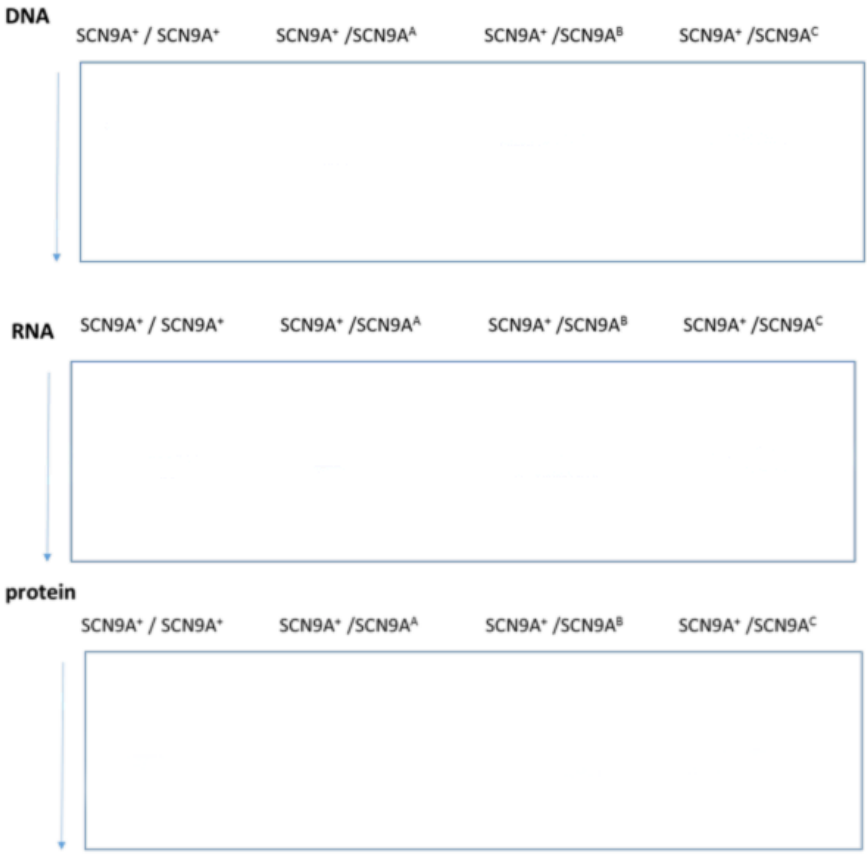
iv. $SCN9A^{B}/SCN9A^{C}$
v. $SCN9A^{A}/SCN9A^{C}$

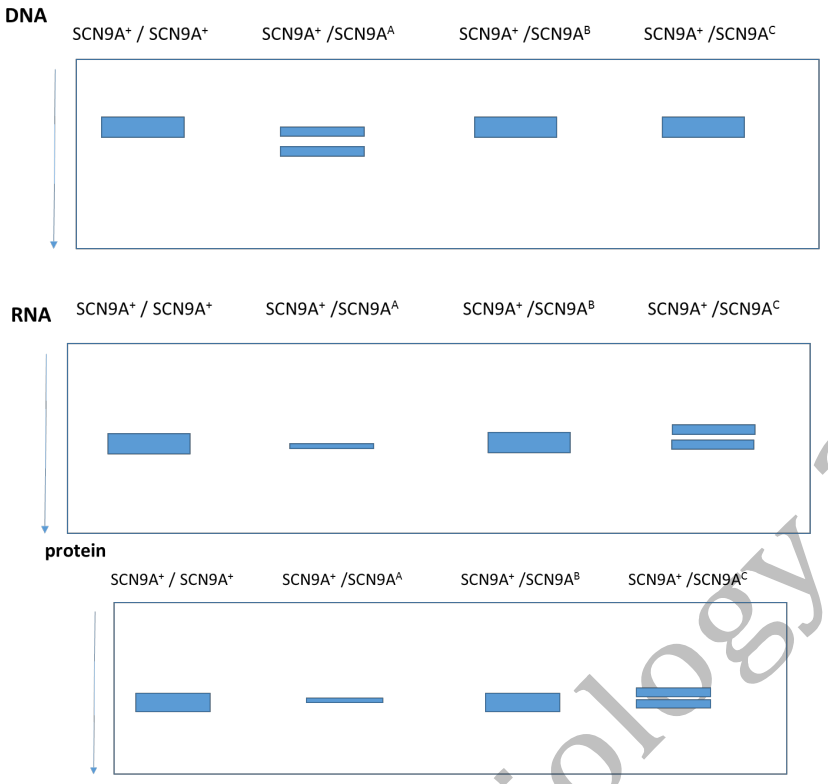
i. $SCN9A^{+}/SCN9A^{A}$ *wild-type*
ii. $SCN9A^{+}/SCN9A^{B}$ *EPD (or wild-type)*
iii. $SCN9A^{+}/SCN9A^{C}$ *wild type*
iv. $SCN9A^{B}/SCN9A^{C}$ *EPD*
v. $SCN9A^{A}/SCN9A^{C}$ *CIP (or EPD)*

These answers depend on the assumptions you made about the effects of the various mutations.

c. Draw the resulting bands on a gel for the DNA, RNA, and protein for the following individuals:

$SCN9A^{+}$ / $SCN9A^{+}$
$SCN9A^{+}$ /$SCN9A^{A}$
$SCN9A^{+}$ /$SCN9A^{B}$
$SCN9A^{+}$ /$SCN9A^{C}$





If $SCN9A^{A}$ causes an insert then yes we should see 2 bands in DNA for $SCN9A^{+}/SCN9A^{A}$ but the top band should be higher than w tie shift those two bands up higher…I couldn't change this figure easily

Note, if your drawings differ from these first look at:

- *How do your predictions about the effects of the mutation compare to mine?*
- *Look at the predictions I made about the effects of the mutation – do they connect to these drawings? How?*