**Question 1 (30 points)**

Gregor Mendel's immortal experiments on plant hybridization made extensive use of variations in seed color and in seed shape.

For example:

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Parents:  round seeded plants x    wrinkled seeded plants
                                ↓
F1:                        round-seeded   x    round-seeded
                                          ↓
F2:                                3 round : 1 wrinkled
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On the basis of these data, Mendel deduced that discrete particles of inheritance (that we now call alleles) are carried by the F1 plants, that the particles reach a compromise for the life of the F1 plant, and then follow his 1st law – equal segregation – to generate the F2 progeny. This was quite revolutionary – for a period of science when people believed in "blending inheritance."

Come to think of it, "seed wrinkling vs not" could be in the eye of the beholder. Surely some seeds are more sunlit when in the pod when others, and then shrink a little it? Maybe the F1 seeds are just a tiny bit wrinkled (as per the "blending inheritance" model), and then all of the phenotypic distribution in the F2 could simply be the "norm of reaction" of the F2 to the amount of sun the seeds are getting? Maybe seed shape exhibits variable expressivity?

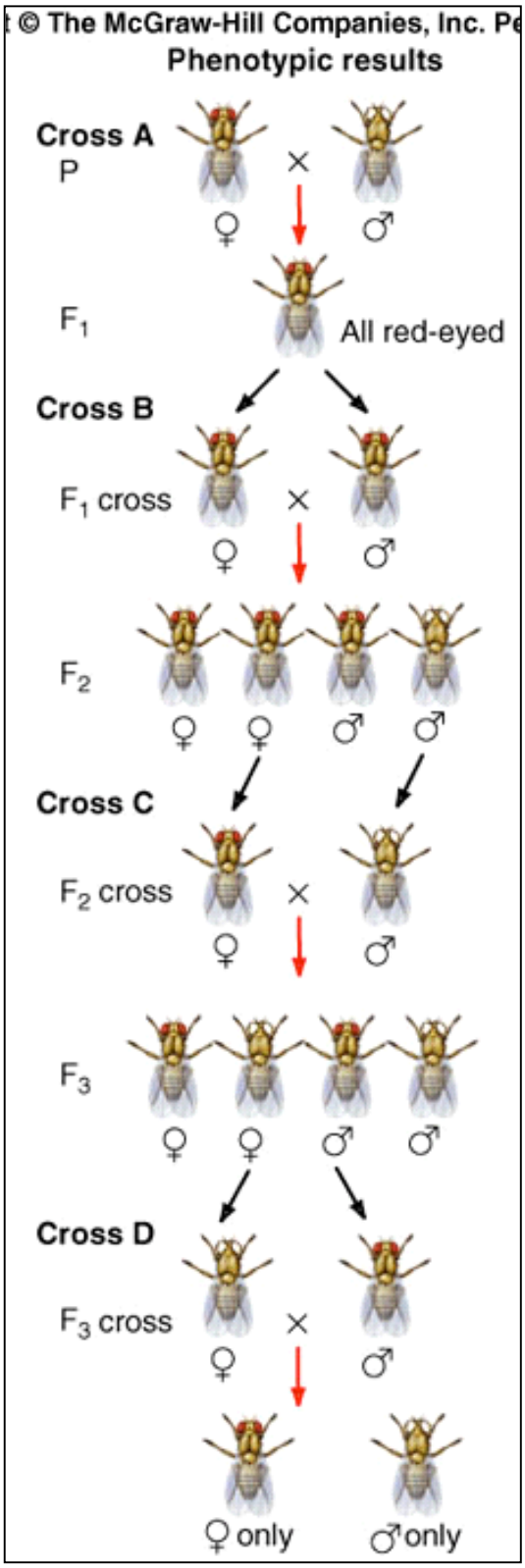
There are, in fact, 3 distinct (i.e., separate) lines of evidence in Mendel's data that argue strongly against this interpretation. The 3:1 phenotypic ratio one sees in the F2 is unequivocally the result of the distinct genetic constitution of the F2 generation, and not an environmental effect on a genetically uniform population – as Mendel's experimental layout and data clearly show. In one-two sentences each, not more, please describe those three separate lines of evidence – as they relate specifically to seed shape.

*This is a harder question that it seems, and many students undoubtedly misunderstood it, having not given sufficient thought to what was being asked. How did Mendel know, that he was NOT working with an environmentally sensitive, variably expressive trait, acting in a genetically uniform population?*

1. *The phenotyping split was always exactly 3:1, which one would not expect from a variably expressive trait.*
2. *Mendel did further crosses on the F2 plants, and found that exacly half of them gave 3:1 progeny, while the other half gave uniform progeny when selfed. This would not have happened if there was nothing but variable expressivity*
3. *The biology of the plant argues against an environmental effect – the 3:1 split is seen with peas that reside in the same pod!*

**Question 2 (30 points)**

A series of experiments by Thomas Hunt Morgan, and his student, Calvin Bridges, on eye color inheritance in *Drosophila*, form the centerpiece of the "chromosomal fact of inheritance." A summary of crosses performed by Morgan is shown below.




This is hypothesis-driven science at its best. Write out the central hypothesis that T.H. Morgan pursued in his studies (one short sentence) – 10 points

*Morgan hypothesized that the gene that specifies red eye color resides on the X chromosome*

*Answers such as "genes reside on chromosomes" get 5 points. Answers such as "eye color is sex-linked" get 7 points.*

Now, pick one cross (just one) of all the ones shown on the left, write it out here (do not use genetic notation, just verbal descriptions of flies and their pedigrees), and explain, what specific consequence of his hypothesis Morgan was testing in this particular cross, and how the data from the cross supported the hypothesis. That part of the answer should take the form "if it is, in fact, true that …, then one would expect that …" (20 points)

*A sample answer (several such answers are possible, and will be correct):*

*Cross C was between a red-eyed female, herself the daughter of a white-eyed male and a red-eyed female, and a white-eyed male. If Morgan's hypothesis is true, then one expects half the males and half the females from this cross to be white-eyed.*

**Question 3 (30 points)**

In class, we discussed the following 3-point testcross done by Alfted Sturtevant: two *Drosophila* were mated: a red-eyed fly that lacked a cross-vein on the wings and had snipped wing edges to a vermilion-eyed, normally veined fly with regular wings. All the progeny were wild type. These were testcrossed to a fly with vermilion eyes, no cross-vein and snipped wings. Sturtevant observed 1448 progeny in 8 phenotypic classes, as shown on the left. Sturtevant then used these data to map the genes, which are, of course, nicely linked.

| | |
|---|---|
| $v \cdot cv^+ \cdot ct^+$ | 580 |
| $v^+ \cdot cv \cdot ct$ | 592 |
| $v \cdot cv \cdot ct^+$ | 45 |
| $v^+ \cdot cv^+ \cdot ct$ | 40 |
| $v \cdot cv \cdot ct$ | 89 |
| $v^+ \cdot cv^+ \cdot ct^+$ | 94 |
| $v \cdot cv^+ \cdot ct$ | 3 |
| $v^+ \cdot cv \cdot ct^+$ | 5 |
| | 1448 |

Many other *Drosophila* species exist, and many are the object of investigation. You decide to take on genetic mapping in *Drosophila virilis*, which appears to be closely evolutionarily related to *D. melanogaster* – in fact, you find that the same recessive mutations (vermillion, no cross-vein, cut wing edges) exist in this species. You perform the same cross that Sturtevant did, but using *Drosophila virilis*: a red-eyed fly that lacked a cross-vein on the wings and had snipped wing edges to a vermilion-eyed, normally veined fly with regular wings. Not surprisingly, all the progeny are wild type. You testcross them to a fly with vermilion eyes, no cross-vein and snipped wings, and are absolutely stunned to discover the proverbial "something completely different." In analyzing 1,000 progeny from this cross, you find the following animals in your vials:

| | |
|---|---|
| Red eyes, no cross-vein, cut wing edges | 395 |
| Vermillion eyes, normal cross-vein, normal wings | 405 |
| Wild-type | 98 |
| Vermillion eyes, no cross-vein, normal wings | 102 |

Once you've recovered from the emotional shock of having seemingly disproven a central tenet of genetics, you do two things (not necessarily in that order):

1. Come up with a hypothesis that explains the striking difference in the mapping data between *D. melanogaster* and *D. virilis* (10 points)

*The* ct *and* cv *genes appear to have relocated very closely to each other in D. virilis (any form of statement that communicates the fact that the two genes are directly next to each other gets full credit)*

2. Map – to the best of your ability – the genes in the *D. virilis* genome. Show your work. (20 points)

*v – 20 mu – ct/cv*

*(cannot determine order of the latter two from the data)*

**Question 4 (10 points)**

You have two haploid yeast strains, one with a mutation at locus A and one with a mutation at locus B. The two loci are on different chromosomes. You mate the strains to form a diploid zygote, then sporulate (force the diploid to undergo meiosis) to form haploids. You randomly select 1000 of these haploid progeny, grow them up into cultures, and do a DNA-based experiment to test the genotypes. You are surprised to find that they do not obey Mendel's second law. Instead of seeing 25% of each possible genotype, you see that a third of the progeny are wild-type, a third have only the A mutation, and a third have only the B mutation. Propose a model to interpret these data which incorporates ONLY the A and B mutations and NO OTHER aberrations. Don't forget that the strains are haploid!

*Answer: The combination of A and B is lethal. Any strain inheriting both mutations has died. The remainder of the genotypes appear at equal frequency in the progeny.*

**Question 5 (20 points)**

George Beadle and Edward Tatum studied *Neurospora* strains carrying mutations that affected different stages of the arginine biosynthesis pathway. You get ahold of one of their Class II mutants, which can grow on minimal media supplemented with late precursor molecules (ornithine, citrulline, or arginine) but cannot grow on minimal media supplemented with precursor molecules which are used to make ornithine, *i.e.* which are upstream of ornithine in the pathway. In this strain, you introduce a mutation in the enzyme that converts citrulline to arginine. You then use some tricks, which are not important here, to mate your double mutant to the original Beadle and Tatum single mutant, creating a diploid. Assuming a model in which each mutation is equivalent to a deletion of the gene encoding an enzyme, what growth behavior do you expect from this diploid on:

(a) Minimal media supplemented with precursors upstream of ornithine
(b) Minimial media supplemented with citrulline.

*Answer: (a), diploid cannot grow. (b), Diploid can grow.*

**Question 6 (30 points)**

In class, we discussed four experiments that discovered conjugation in bacteria (Lederberg), its unidirectional nature (Hayes), the "spaghetti hypothesis" of the genome being threaded from one bacterium to another (Lwoff and Jacob/Monod), and phage-based transfer of genes from one bacterium to another, i.e., transduction (Zinder and Lederberg).

Consider a novel, pathogenic strain of E. coli that appears to be resistant to amoxicillin (a commonly used antibiotic that kills wild-type E. coli)

Describe, in a numbered list format, how you would … (10 points each)

A. … confirm that $amo^R$ is an actual gene in the E. coli genome, i.e., that resistance to amoxicillin is genetic.

*The L-D experiment in its direct form, if done on the resistant bacteria, is inapplicable – all cultures will yield a large number of resistant colonies. Three possible answers:*

| | | |
|---|---|---|
| *L-D on the sensitive bacteria*<br>*1. Take wt bacteria (start clonal and pool cultures)*<br>*2. Expose to amo*<br>*3. Count number of resistant colonies*<br>*4. Compare* | *Direct genetic transfer – via conjugation or transduction*<br>*1. Mate resistant and sensitive bacteria (or infect resistant with phage)*<br>*2. Score for acquisition of resistance* | *Amo "washout"*<br>*1. Culture resistant bacteria for an extended period of time in non-amo medium*<br>*2. Expose to amo*<br>*3. Number of resistant colonies should not drop significantly* |

B. … map the location of the $amo^R$ gene on the E. coli chromosome relative to the leu genetic marker (the same one that Lederberg used). You are free to assume the existence of any bacterial strains or other reagents and biological materials you wish, but for your answer to be considered correct, you must explicitly state that you are assuming that they exist. (20 points)

*Do an interrupted mating experiment with a $leu^+$ $amo^R$ bacterium and a $leu^-$ $amo^S$ bacterium.*

C. … prove that the $amo^R$ gene you have just identified and mapped is sufficient to endow resistance to amoxicillin to a strain of E. coli that, for unknown reasons, does not engage in conventional F-factor based conjugation, but appears to be a normal bacterium in every other respect.

*Infect the $amo^{R-}$ bacterium with lytic phage, take the phage supernatant, and infect the other bacterium with that phage, then plating those infected bacteria on amoxicillin under conditions that favor phage latency. Some students will propose a recombinant DNA-based way to do this, which is fine.*