Breeding organisms with a Specified Genotype

Objective: By the end of this exercise students should be able to design a series of genetic crosses to produce an organism with a specified, homozygous autosomal recessive genotype.

Skills: The students will need to use their knowledge of Mendelian genetics - specifically F_1 and F_2 crosses - and probability to accomplish the breeding this task.

How I have used these problems: In the many years that I have taught genetics, I have observed that many students are quite good at completing monohybrid and dihybrid crosses when the parents (i.e., the P_1 generation) are specified and the crosses are presented as a distinct problem (e.g., PIn a cross between AAbb and aaBB individuals, what are the expected phenotypes and their ratios in the F_2generationΠ). Similarly, they can estimate the probability of getting a particular genotype or phenotype in any specified generation when presented with specific parental genotypes..

Some students, however, have difficulty using their knowledge of genetics to do PusefulΠ work. When these students are presented with a simulation of a real life problem - breeding an organism with a specified genotype - they have difficulty with choosing parents, deciding which crosses to do and in what order, doing more than two crosses, and using probability estimates to estimate the scope of the work involved. They are also uncomfortable with "discarding" unused progeny.

Another issue that is minor in scope but has real implications for students is that all organisms have all genes *even if those genes are not relevant for the problem*. In the following problems, I ask students to manipulate only three out of four genes to assess their ability to correctly write out the entire genotype.

I have used these problems as quizzes, homework problems and as jigsaws in recitation sections. I prefer the jigsaw approach since each student has to explain his/her answer and they get to see that there is more than one correct answer.

Extra credit: I use the extra credit question to assess the elegance of a studentMs answer. It is my experience that students will try to do the experiment in the least possible time and will spend the smallest amount of money. However, these decisions give a chance to discuss probability as it relates to obtaining a pair of organisms with the desired genotypes.

If your students need an example, there is one online at
gen.rutgers.edu/constructing a homozygote for more.htm

Answer for one question (Form C). All other problems are solved using the same logic.

Known Information - Given in the set up of the problem.	
Genetics:	*Purple* eye is dominant to *blue* eye (A > a) *Striped pellicle* is dominant to *solid* pellicle (B > b) *Two terminal claws* is dominant to *four terminal claws* (C >c) *Green* pellicle is dominant to *white* pellicle (D > d) All four genes are autosomal and on different chromosomes. The four mutations **blue-eyes, solid colored pellicle, white pellicle** and **four terminal claws** are completely recessive.
Available Stocks:	**There are only 5 available stocks (below) . All stocks are completely homozygous.** **For stocks 2 through 5, only the mutant phenotype is described,.** **Strain 1 is wildtype: The Phenotype is : Purple Eye, striped, green pellicle and two terminal claws** **Strain 2 has blue eyes instead of purple eyes.** **Strain 3 has a solid pellicle instead of a striped pellicle.** **Strain 4 has four terminal claws.** **Strain 5 has a white pellicle**

The Assignment:

Using the some or all of the stocks provided, create a homozygous stock of **blue-eyed, solid, green** dust bears with **two terminal claws. You must hand your professor at least 20 parents (10 males and 10 females) to complete the assignment.**

ANSWERS

A. What are the genotypes of the 5 homozygous strains?

Strain 1	AABBCCDD	Strain 2	aaBBCCDD
Strain 3	**AAbbCCDD**	Strain 4	**AABBccDD**
Strain 5	**AABBCCdd**		

B. What is the genotype of the homozygous line that you are expected to make?

The target genotype is **aabbCCdd**

C. One possible answer.

The first target is to obtain at least one male and at least one female with the genotype**aabbCCDD**. There are three possible starting crosses. I chose as parents a female with blue eyes from strain 2 and a solid male from strain 3. These are crossed to produce an F_1 generation.

Cross No.	Generation	Female		Male
1				
	P_1	**aaBBCCDD (blue eyes)**	**X**	**AAbbCCDD (solid)**
	F_1	**All F_1 have a completely wildtype phenotype with genotype AaBbCCDD. A single pair of dust bears is intercrossed to produce an F_2 generation.**		
2				
	F_1	**AaBbCCDD**	**X**	**AaBbCCDD**

In the F_2 generation there will be 9 different genotypes. These will fall into four phenotypic classes in a 9:3:3:1 ratio.

Progeny Phenotype	**Exp. Freq.**	**Exp. No. females**	**Exp. No. males**
Purple Eye, striped, green pellicle and two terminal claws	**9/16**	**36**	**36**
Blue Eye, striped, green pellicle and two terminal claws	**3/16**	**12**	**12**
Purple Eye, **solid**, green pellicle and two terminal claws	**3/16**	**12**	**12**
Blue Eye, solid, green pellicle and two terminal claws	**1/16**	**4**	**4**

1/16 of the progeny *are expected to* have the doubly recessive phenotype **blue eye, solid (with the wildtype phenotype of green pellicle and two terminal claws)** and have the corresponding genotype **aabbCCDD.**

Design Considerations: In any breeding experiment the constraints are time and money. When attempting to get a rare genotype, such as the doubly recessive homozygote **aabbCCDD**, there is always the possibility that individuals with the desired genotype might not show up by chance alone. If that happens, all the crosses will have to be redone. Generally, geneticists tend to set up more crosses than they need. It is often better to spend money than to waste time.

In this case, since the dust bears always produces 64 males and 64 females, the chance of **not** obtaining a male with **Blue Eye, a solid** green pellicle and two terminal claws is about 1.6%. The same is true of a female with the same phenotype. Since you only need either a single male *or* a single female of the desired genotype to continue the series of crosses, one F_2 cross should be sufficient.

The next step is to cross the homozygous **dust bear with blue eyes, a solid** green pellicle and two terminal claws dust bear (**aabbCCDD**) to a homozygous dust bear (AABBccDD) with four terminal claws. I will assume that the dust dear with **blue eyes, a solid** green pellicle and two terminal claws is female. A single cross will yield 64 males and 64 females in the F_1 generation.

Note: Dust bears with **blue eyes, a solid** green pellicle and two terminal claws that are not used in Cross 3 might be intercrossed to establish a homozygous line (genotype aabbCCDD). If something goes wrong in either Cross 3 or Cross 4, the student will have saved two days of work.

Cross No	**Generation**	**Female**		**Male**
3				
	P_1	**aabbCCDD (blue&solid)**	**X**	**AABBccDD (four claws)**
	F_1	**All F_1 have a completely wildtype phenotype the genotype with genotype AaBbCcDD. Many pairs (see below) of dust bears are intercrossed to produce an F_2 generation.**		
4				
	F_1	**AaBbCcDD**	**X**	**AaBbCcDD**

Genotypes in the F_2 Generation:

The F_2 progeny have should have 27 genotypes and 8 phenotypes

Expected Progeny Phenotypes	**Exp. Freq.**	**Exp. No. females**	**Exp. No. males**
Purple Eye, striped, green pellicle and two terminal claws	**27/64**	**27**	**27**
Blue Eye, striped, green pellicle and two terminal claws	**9/64**	**9**	**9**
Purple Eye, **solid**, green pellicle and two terminal claws	**9/64**	**9**	**9**
Blue Eye, solid, green pellicle and two terminal claws	**3/64**	**3**	**3**
Purple Eye, striped, green pellicle and **four** terminal claws	**9/64**	**9**	**9**
Blue Eye, striped, green pellicle and **four** terminal claws	**3/64**	**3**	**3**
Purple Eye, **solid**, green pellicle and **four** terminal claws	**3/64**	**3**	**3**
Blue Eye, solid, green pellicle and **four** terminal claws	**1/64**	**1**	**1**

Design Considerations: In this case, since the dust bears always produces 64 males and 64 females, the chance of **not** obtaining a male with **Blue Eye, a solid** green pellicle and **four** terminal claws is about 36.5%. The same is true of **not** obtaining a female. Since you need ***both*** a male and a female to establish the line, this is not a place to skimp on the number of F_2 crosses (Cross 4).

Each F_2 cross is an independent event. The chance of setting up two crosses and not getting a single male with **blue eyes, a solid** green pellicle and **four** terminal claws is .365*.365 = .13 or 13%. This probability is still unacceptable. With 5 crosses the chance of **not** obtaining a male with **t**he desired genotype is less than 1%. Since I would have invested fours days at this point, I personally would double the number of crosses to 10.

Cross 5: From among the F_2 progeny choose all the dust bears which have **blue eyes, a solid** green pellicle and **four** terminal claws. These should be intercrossed. For each female included in the cross the student will obtain 64 females and 64 males with the desired phenotype. Ten pairs of these can be handed to the professor.

Save the Other Stocks.

Among the F_2 progeny are three other important homozygous lines:		
	a.	**Blue Eye, solid,** green pellicle and two terminal claws (already established from Cross 2)
	b.	**Blue Eye**, striped, green pellicle and **four** terminal claws
	c.	Purple Eye, **solid**, green pellicle and **four** terminal claws
If this were a lab situation, the above dust bears might be intercrossed to establish homozygous lines for further study.		

Time and cost budget.

a. If every single cross produced a male a female of the desired genotype the assignment can be completed in 5 days for a total cost of $1.25.

b. Using the prudent approach (2 replicates of Cross 2 and 10 replicates of cross 4) assignment can be completed in 5 days for a total cost of $3.75.

c. Using the brute force approach (see #1 **Variants and Common mistakes- below)** it can be done in 4 days for a cost of between $6.00 to 16.00.

Variants and Common mistakes:

1 Some students set up 20 to 64 F_2 crosses **(Cross 4).** They are extremely likely to obtain 20 males and 20 females from among the 1280 - 4096 progeny of each gender. This costs more money but saves a day.

2. Some students set up the initial cross as if each dust bear had only one gene. That is, Cross 1 is written as **aa x bb.** The F_1 progeny then have the genotype **ab.** At this point the students are stuck.

I rewrote the original question so that students had to specify the target genotype and the genotypes of the five laboratory strains. This reduced the number of students with this misunderstanding. Students who work in groups are corrected by their peers. If this is used as a quiz or homework problem, there is no easy fix. When a student is this badly "off track", I need have him/her come to my office to step through the problem.

3. I**nventing dust bears that don't exist.** I occasionally have students who complete Cross 3. They then take the triple heterozygote and backcross it to dust bears that have **blue eyes, a solid** green pellicle and **four** terminal claws. I gently point out that if they had such a strain, that there would not be any reason to do this exercise. A few students still don't understand the problem and need have to come to my office to step through the problem.

4. **Mendelian ratios are rules.** Some students believe that Mendelian ratios are fixed laws. That is, in the F_2 generation resulting from cross 2 there must be exactly 4 males and 4 females of the desired genotype and that in the F_2 generation resulting from Cross 4, there must be exactly 1 male and 1 female of the desired genotype. They get full credit for the genetics principles but lose points on the practicality of their solution. A review of probability with the student is required.