

Constructing a homozygote for more than one gene

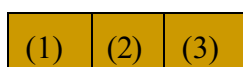
For every gene with two alleles there are two homozygous genotypes and one heterozygous genotype. For each independently assorting gene associated with a phenotype, the number of completely homozygous genotypes increases geometrically. However, such homozygous genotypes, make up a smaller percentage of the total genotypes.

Number of Genes Associated with the Phenotype	Number of Genotypes	Number of Homozygous Genotypes	Percentage of Homozygous Genotypes
1	3	2	66.7%
2	9	4	44.4%
3	27	8	29.6%
4	81	16	19.8%
5	243	32	13.2%
n	3^n	2^n	$2^n/3^n\%$

Completely homozygous genotypes are desirable since it is possible to set up a stock of organism that breeds true every generation. These organism homozygous for multiple genotypes have to be "constructed" from a series of genetic crosses. Such construction is often done using two genes at a time.

Example: Flag beetles have a box on their back which is divided into thirds (see below). The color in each box is normally **beige** (i.e., beige is wildtype). Three recessive genes are known which color a different third of the box: **Kelly green** (1), **pure white** (2) and **orange** (3). These genes are independently assorting.

Box on back of normal beetle:



Genetically:

Gene 1	Brown is dominant to green	(G > g)
Gene 2	Brown is dominant to white	(W > w)
Gene 3	Brown is dominant to Orange	(O > o)

In the lab, there are four strains of Flag beetles which *breed true*:

Strain 1: Brown (all three sections).

Strain 2: Green (section 1, other two sections are Brown).

Strain 3: White (section 2, other two sections are Brown).

Strain 4: Orange (section 3, other two sections are Brown).

The Question: A certain Genetics professor decides to breed a special beetle which looks like the Irish Tricolor Flag (Green, White and Orange). How does he do this?

Desired Target Phenotype: Box on back of *Irish Tricolor* beetle:



Material at Hand:

There are four homozygous strains available in the lab.

<u>Phenotype</u>	<u>Genotype</u>
Brown	GGWOO
Green	ggWOO
White	GGwwOO
Orange	GGWWoo

The Desired Genotype of a Homozygous *Irish Tricolor* beetle

The target Irish Tricolor Flag beetle needs to have genotype ***wwggoo*** so that it will express the three different colors simultaneously. This genotype is approached through a number of steps.

Step one is to construct a double homozygote. This double homozygote can be either white/green, white/orange, or green/orange (the other section of the box would be brown)

The professor decides to construct a **green/orange** beetle first (that is he works with genes 1 and 3 and ignores gene 2)





His target is a homozygous flag beetle with genotype **ggWW00** and phenotype green brown and orange (seen below)



The crosses required to get the desired genotype			
P₁	ggWWOO (green)	x	GGWWoo (orange)
F₁	GgWWOo (wildtype)	x	GgWWOo (wildtype)

In the F₁ generation, he gets all wildtype (entirely beige) beetles which must have the genotype **GgWWOo**.

The F₁ beetles are intercrossed to make an F₂ generation. In the F₂ generation he gets 9 different genotypes for gene 1 and 3. These fall into four phenotypic classes brown, **green**, **orange**, **green & orange** in a 9:3:3:1 ratio.

Beetle Box	Phenotypic description frequency.	Phenotypic frequency.
	Brown	9/16
	Green	3/16
	Orange	3/16
	Green and orange	1/16

Thus, 1/16 of the progeny will have the doubly recessive phenotype **green & orange** and have the corresponding genotype **ggWWoo**. (Note: the professor might have to carry out this cross a number of times to get enough males and females to establish a viable, pure breeding **green& orange** population of Flag beetles.

F₂ Generation:

Gametes	GWO (1/4)	GWo (1/4)	gWO (1/4)	gWo (1/4)
GWO (1/4)	GGW ^W OO (1/16)	GGW ^W Oo (1/16)	GgW ^W OO (1/16)	GgW ^W Oo (1/16)
GWo (1/4)	GGW ^W Oo (1/16)	GGW ^w oo (1/16)	GgW ^W Oo (1/16)	GgW ^w oo (1/16)
gWO (1/4)	GgW ^W OO (1/16)	GgW ^W Oo (1/16)	ggW ^W OO (1/16)	ggW ^W Oo (1/16)
gWo (1/4)	GgW ^W Oo (1/16)	GgW ^w oo (1/16)	ggW ^W Oo (1/16)	ggW^woo (1/16)

Step 2: Now that the professor has a homozygous **green & orange** strain established (section 2 is still brown) he can cross individual beetles from that strain with individual beetles from the homozygous **white** strain (**GGwwOO**) that he has in the lab.





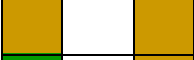
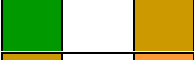
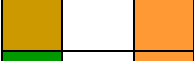
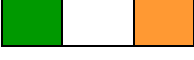
This crosses produces a triple heterozygote in the F₁ generation (**GgWwOo**). He then intercrosses the F₁ progeny to produce an F₂. He will then get the desired homozygous IRISH TRICOLOR flag beetle in a very low frequency.

The crosses required to get the desired genotype			
P ₁	ggWWoo (green & orange)	x	GGwwOO (white)
F ₁	GgWwOo (wildtype)	x	GgWwOo (wildtype)

Genotypes and Phenotypes in the F₂ Generation:

The F₂ progeny have 8 phenotypes (**Brown, white, green, orange, white&green, white&orange, green&orange** and **Irish Tricolor (white&green&orange)**) and 27 genotypes for genes 1, 2 and 3. The phenotypes are illustrated below.

Tricolor should occur with a frequency of 1/64. The professor might have to repeat this cross a number of times to get enough Tricolor beetles to establish a new pure breeding lines (say 10 pairs of males and females).

Phenotypes			
Beetle Box	Phenotypic description		frequency.
			Phenotypic frequency
	Brown		27/64
	Green		9/64
	Orange		9/64
	Green and orange		3/64
	White		9/64
	Green and White		3/64
	White and Orange		3/64
	Irish Tricolor		1/64

Genotypes in the F₂ Generation

	GWO (1/8)	GWo (1/8)	gWO (1/8)	gWo (1/8)	GwO (1/8)	Gwo (1/8)	gwO (1/8)	gwo (1/8)
GWO (1/8)	GGWwO O	GGWwO o	GgWwO O	ggWwOo	GGWwO O	GGWwO o	GgWwO O	GgWwOo
GWo (1/8)	GGWwO o	GGWwo o	GgWwO o	ggWwOo	GGWwO o	GGWwo	GgvOo	GgWwoo
gWO (1/8)	GgWwO O	GgWwO o	ggWwOo	ggWwOo	GgWwO O	GgWwOo	ggWwOo	ggWwOo
gWo (1/8)	ggWwOo	GgWwOo	ggWwOo	ggWwOo	GgWwOo	Ggvoo	ggWwOo	ggWwoo
GwO (1/8)	GGWwO O	WwGGO o	GgWwO O	GgWwOo	GGwwO O	GGwwOo	GgwwOo	GgwwOo
Gwo 1/8)	GGWwO o	GGWwoo	GgWwOo	GgWwoo	GGwwOo	GGwwoo	GgwwOo	Ggwwoo
gwO (1/8)	GgWwO O	GgWwOo	ggWwOo	ggWwOo	GgwwOo	GgwwOo	ggwwOo	ggwwOo
gwo (1/8)	GgWwoo	GgWwoo	ggWwOo	ggWwoo	GgwwOo	Ggwwoo	ggwwOo	ggwwoo (1/64)

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