Genetic mapping and manipulation: Chapter 8-Dominant mutations*

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1. Basics

Dominant alleles were first described by Mendel to account for the patterns he observed with respect to flower color. For example, red flowers are produced when the R allele is present in one or more copies (i.e., genotypes **R/r** and **R/R**), versus white flowers, which are produced only when the **r** allele is homozygous (**r/r**). In this case, the **R** allele is said to be dominant to the **r** allele, which is recessive. As the study of genetics has matured, the common definition of ***dominance*** has come to refer to alleles whose phenotype is manifest when present as a heterozygote (**R/r**). Dominant alleles may also be phenotypically deterministic when present along with two or more recessive alleles (e.g., **R/r/r**), a situation sometimes encountered in transgenic strains or with free duplications.

A good example of a dominant allele in *C. elegans* is the ***rol-6(su1006)*** allele, which causes a roller (**Rol**) phenotype. ***rol-6(su1006)*** animals exhibit the **Rol** phenotype when they are of the following genotypes: ***rol-6/rol-6; rol-6/+;*** or +/***rol-6***. Because the **Rol** phenotype is observed when a single mutant copy of ***rol-6(su10060)*** is present,

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the ***rol-6(su1006)*** allele is said to be dominant. Keep in mind that not all alleles of a particular gene will be dominant; there are several ***rol-6*** alleles that exhibit recessive phenotypes. Dominance or recessivity are allele-specific properties. They are not gene-specific properties.

2. Isolating dominant alleles

Depending on the particular developmental question in which you are interested, the systematic isolation of dominant alleles may be desirable. If you decide this is the case, the isolation of dominant alleles is straightforward. Although the typical genetic screen in *C. elegans* often aims to isolate recessive, loss-of-function alleles, as shown in Figure 1, the isolation of a dominant mutation requires one to simply screen the F1 generation (i.e., the self-progeny of mutagenized P0 worms), as shown in Figure 2.

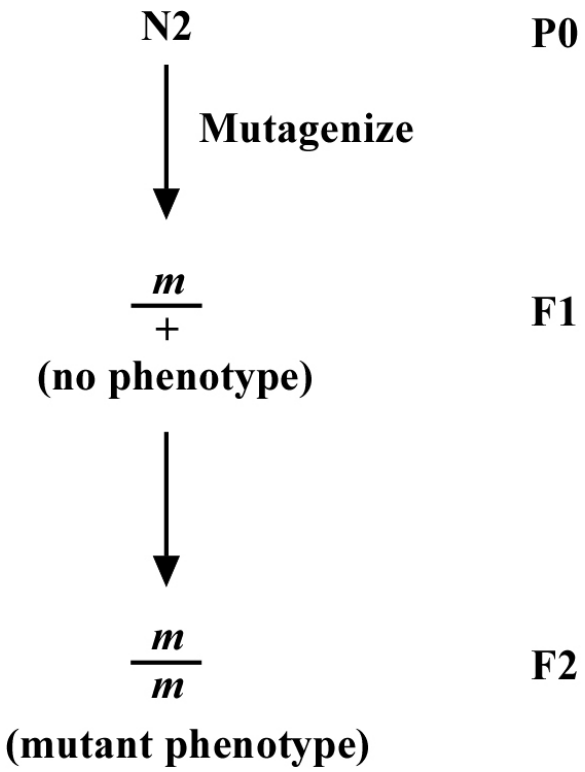

Figure 1.

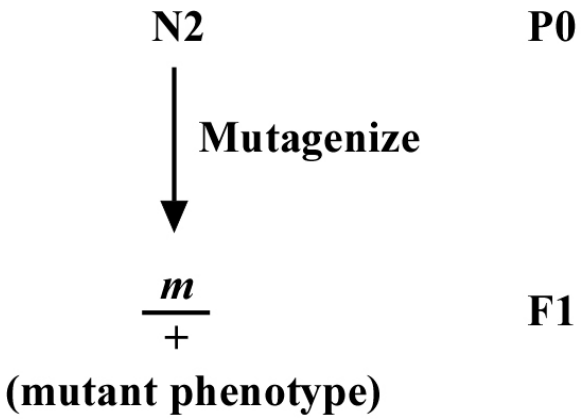

Figure 2.

This is trivial. One can also isolate dominant alleles in the first screen, because dominant mutations will exhibit the mutant phenotype in the F2 generation as well as the F1 generation. In a non-clonal screen, where F2 worms on a plate will derive from several different P0 animals, one may not notice that a particular allele is dominant until outcrossing fails to eliminate the mutant phenotype in cross-progeny. When outcrossing any newly isolated mutation, one should carefully observe the genetic behavior of an allele to determine whether it is dominant or recessive.

3. Mapping a dominant mutation

Whether your dominant mutation was isolated on purpose or by chance, the next step will be to map it to a chromosome. You'll recall from earlier sections that recessive alleles are initially crossed to wild type and then progeny of the heterozygous animals are then scored for segregation of the mutations and known genetic markers. If an allele is dominant, however, it is necessary to change our thinking slightly when scoring the segregation of phenotypes in mapping strains. Because we cannot determine whether a phenotypically mutant animal is heterozygous or homozygous for the dominant allele by simple observation, we use the alternative strategy of mapping the absence of our dominant allele. Once one has thought about it carefully, it can often be easier to map true dominant mutations than recessive mutations.

The phenotype of a recessive mutation disappears when crossed into a mapping strain. Consider ***lin-1***, which causes a multivulval (**Muv**) phenotype when crossed into a strain as shown in Figure 3. We can then score the co-segregation of ***lin-1*** and ***dpy-17 unc-32*** in the normal manner by picking **Muv non-Dpy Uncs** and noting how often the **Dpy Unc** phenotypes co-segregate with ***lin-1*****Muv**.

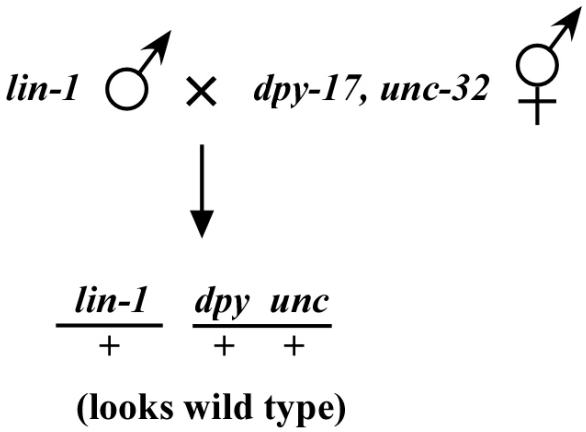

Figure 3.

Now consider a dominant mutation that, in contrast to ***lin-1***, exhibits the mutant phenotype when the allele is present as a heterozygote. When we cross into our chromosomal mapping strains, all the heterozygous cross progeny will exhibit the mutant phenotype. Let's consider an imaginary dominant mutation, ***dom-1***, as shown in Figure 4, which we'll say causes a spiked-head phenotype. These ***dom-1/+*** heterozygotes will display the spiked-head phenotype and will be indistinguishable from ***dom-1*** homozygotes. Thus, we will be unable to score the segregation of ***dom-1*** with ***dpy-17***, ***unc-32*** by following spiked-head animals because we won't know whether the animals are ***dom-1/+*** or ***dom-1/dom-1***.

The trick to mapping such true dominant mutations is to follow the animals that do not display the dominant phenotype. In other words, ignore the ***dom-1*** phenotype and look only for those animals that are wild type. The reason for this is as follows: as we can always tell when ***dom-1*** is present because of the dominant spiked-head phenotype, we follow the absence of ***dom-1*** and note how often the markers segregate with **non-spiked-head** animals.

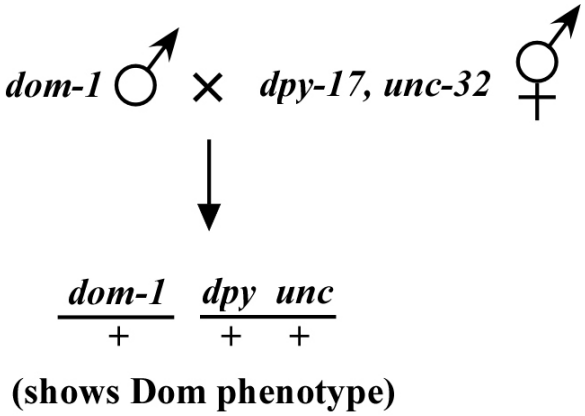

Figure 4.

The results from chromosomal mapping of dominant mutations are apparent in the F2 generation. Because you are following the absence of ***dom-1***, at this stage you are looking for **non-spiked-head** animals. If ***dom-1*** is on the same chromosome as your markers and is relatively close (as shown in Figure 5; case #1), then the only animals with normal heads will be the **Dpy Uncs**. Also if you were to pick **spiked-head** animals and look for those that throw 100% spiked-head animals in their progeny (***dom-1/dom-1***), few or none will throw **Dpy Uncs**. If, however, ***dom-1*** is on a different chromosome from the markers (as shown in Figure 5; case #2), then it will segregate independently from the markers, and 3/4 of the **Dpy Unc** progeny will display **spiked heads**. Also in this case, 2/3 of the **spiked-head** (**non-Dpy Unc**) progeny that throw exclusively **spiked-head** progeny (***dom-1/dom-1***) will throw **Dpy Uncs**.

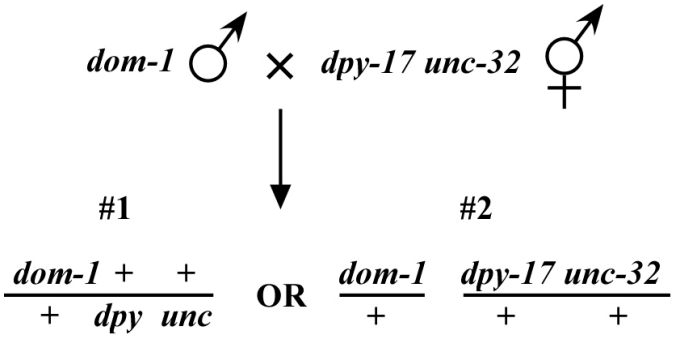

Figure 5.

Once we have our dominant mutation mapped to a chromosome, it is similarly easy to collect data for three-point mapping. We'll start again with our balanced strain that is heterozygous for both ***dom-1*** and our markers. This strain will have the spiked-head phenotype. In this example, we'll assume ***dom-1*** lies between ***dpy-17*** and ***unc-32***. When looking for recombinant **Unc-non-Dpy** or **Dpy-non-Unc** animals, we will know immediately whether or not the recombinant picked up the mutant ***dom-1*** allele because of its dominance. Recombinants that pick up the ***dom-1*** allele will have a spiked head, and recombinants that don't pick it up will have wild-type heads. Quick and easy.

4. Different types of dominant mutations

Why do some mutations act in a dominant fashion? Below we examine some different mechanisms through which a mutation can confer a dominant phenotype. In certain situations, different dominant alleles may require different mapping strategies. These situations must be managed on a case-by-case basis. In each example below, we will consider the fictional ***dom-1*** gene and imagine different situations that could give rise to various types of dominant alleles in ***dom-1***.

4.1. Haploinsufficiency

This describes a situation in which one copy (*haplo*) of a wild-type gene is not enough to provide wild-type function when the other copy is compromised. This can only occur for loss-of-function alleles. Consider again our fictional dominant mutation, ***dom-1***. Let's assume that a certain threshold of ***dom-1*** activity is required to avoid the abnormal spiked-head phenotype; two copies of the wild-type gene are required to achieve that threshold, and any drop below that threshold allows the mutant spiked head to form. Mutations in ***dom-1*** that reduce or eliminate its activity would therefore behave dominantly because in heterozygous animals, the single remaining wild-type copy of the ***dom-1*** gene would be *insufficient* to provide the wild-type levels of gene activity. Thus, the loss-of-function ***dom-1*** mutant allele may produce a similar phenotype whether present in one or two copies and behaves in a dominant fashion. Alternatively, ***dom-1/+*** heterozygous animals may display a phenotype that is quantitatively or qualitatively different from homozygous ***dom-1/dom-1*** animals, since the former would still retain half the normal gene dose.

4.2. Dominant-negative alleles

These typically occur when the mutant allele does not function normally *and* either directly inhibits the activity of the wild-type protein (usually through dimerization) or inhibits the activity of another protein that is required for the normal function of the wild-type protein (such as an activator or downstream component in a pathway). Although this situation can effectively result in the loss of function of the wild-type protein, it differs markedly from haploinsufficiency. Consider an animal that is heterozygous for a dominant-negative allele of ***dom-1***. In this case, we'll also imagine that the single wild-type copy of ***dom-1*** would normally provide enough ***dom-1*** activity to avoid the spiked-head phenotype. However, because of a dominant-negative version of ***dom-1*** would actually ***interfere*** with the function of wild-type ***dom-1*** its activity is further reduced and a mutant phenotype results.

A well-known example of a gene that can incur dominant-negative mutations is the small GTPase Ras. These dominant-negative alleles of Ras are not functional themselves because they preferentially bind GDP and stay locked in the inactive state. In addition, they also prevent the Ras exchange factor (which binds Ras-GDP and catalyzes GDP/GTP exchange and subsequent Ras activation) from acting on wild-type Ras, essentially killing all Ras activity.

4.3. Dominant gain-of-function (GOF) alleles

Also termed hypermorphs, these refer to mutations that result in elevated levels of gene activity. In some cases, dominant GOF mutations may acquire novel biochemical activities, in which case they may be referred to as neomorphs. It is possible to imagine numerous scenarios that might lead to the removal of normal regulatory constraints and the enhancement of protein activity. For example, a mutation in the promoter region could lead to overexpression of the gene and the saturation of negative regulatory pathways. Alternatively, point mutations in a region of a gene important for its regulation could lead to inappropriate activity and mutant phenotypes. Let's revisit ***dom-1*** and imagine it is an enzyme whose activity promotes head development. Assume that normal levels of ***dom-1*** activity result in normal head development and any ***dom-1*** activity above normal levels results in a spiked head. Also assume that a negative regulatory phosphate group is added to an N-terminal serine when ***dom-1*** activity gets to the threshold required for normal development. A point mutation that makes this serine phosphorylation impossible (e.g., Ser → Ala) could remove the negative regulation of ***dom-1*** and allow its activity to proceed unchecked, thus leading to the **spiked-head** phenotype. In short, too much of a good thing can lead to developmental abnormalities.

4.4. Semi-dominant alleles

It is actually quite typical for dominant alleles to behave in a partially dominant fashion. Alleles are designated semi-dominant when the penetrance of the phenotype in heterozygous animals (***dom-1/+***) is less than that observed for homozygous animals (***dom-1/dom-1***). For ***dom-1***, this would be the case if ***dom-1/dom-1*** animals were 100% spiked head and ***dom-1/+*** animals were 60% spiked head. This is an important point, as the basic mapping strategies outlined above were assuming 100% dominance. In practice, this is not necessarily that difficult to deal with, as the presence of the mutation will always be seen by the next generation, as is the case for any recessive allele. Thus mapping a semi-dominant mutation will simply require following progeny for an extra generation to distinguish between ***dom-1/+*** and +/+ animals.

5. Genetic tests for dominance classes

To attempt to distinguish between various classes of dominant mutations, a number of genetic tests can be performed. For example, to determine if a mutant phenotype observed in a heterozygous animal is due to haploinsufficiency, one can directly examine animals that are heterozygous for a chromosomal deficiency that removes the entire gene (as well as a number of other genes presumably). Alternatively, if a deletion or null allele of the gene exists, placing this mutation over the wild-type chromosome could provide an even cleaner answer. In addition, to distinguish haploinsufficieny effects from hypermorphic mutations, one can further compare homozygous mutant animals (***dom-1/dom-1***) with animals that are heterozygous for the mutation and the deficiency (***dom-1/Df***). If the homozygous mutants show a more severe phenotype than the mutant allele over the deficiency, then it is likely that the mutation is at least partially dominant, although one can have both dominance and haploinsufficient effects for the same allele.

In addition, a hypermorphic mutation would be expected to exert an effect even in the presence of two normal copies of the gene. Thus, a genetic test of this can be carried out using a free duplication that contains a wild-type copy of the gene, which is examined in the background of the heterozygous mutant (e.g., ***dom-1/+; Dp***). A further test is to examine ***dom-1/dom-1; Dp*** animals. In this case, if the mutant allele is not hypermorphic (only LOF associated with haploinsufficieny), the phenotype of this animal should be no more severe than ***dom-1/+*** animals and may even be less severe if the ***dom-1*** allele contains some residual activity.

Other questions may be more difficult to answer genetically, particularly in the absence of knowing or understanding the molecular functions of the gene. For example, distinguishing dominant negatives from dominant gain of function alleles may be difficult in a vacuum. The ability of RNAi to phenocopy or enhance a dominant mutation would suggest that the mutation is a dominant negative, although a negative result in this case is difficult to interpret. Also, if the gene is cloned, then attempts to overexpress the wild-type version of the gene product may be informative in this regard, as phenocopy would indicate a hypermorphic mutation. Also, a dominant negative might be expected to be less penetrant in a background that contains one or more copies of the wild-type gene (e.g., ***dom-1/dom-1*** versus; ***dom-1/dom-1; Dp***), although a number of hand-waving explanations can theoretically weaken these types of arguments.

In closing, we refer readers to a number of published papers dealing with various types of dominant mutations in ***C. elegans*** (see below). We hope you enjoyed this discussion of dominant alleles. Now get back to work, dammit (A. Spencer).

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