

Question & Answer

Q&A: EpistasisFrederick P Roth^{*}, Howard D Lipshitz[†] and Brenda J Andrews^{†,‡}**What is epistasis?**

Hmmm. Are you a classical geneticist, a population geneticist, or a medical doctor?

OK, what does a classical geneticist mean by epistasis?

William Bateson coined this term about 100 years ago for a genetic interaction in which one mutation masks or suppresses the effects of another allele at another locus [1].

What do you mean exactly by a genetic interaction?

Two mutations have a genetic interaction when their combination yields a surprising phenotype that cannot be explained simply by the independent effects observed for each mutation alone.

Fine, so what does a population geneticist mean by epistasis?

RA Fisher used 'epistacy' and later 'epistasis' to describe genetic interactions more generally [2]. We think that population geneticists hijacked this term over a decade after its coinage just to confuse the classical geneticists.

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OK, what does a medical doctor mean by epistasis?

A thin film on the surface of a urine specimen. Enough said on that topic.

I'm confused. Epistasis seems to mean genetic interaction under both classical and population genetics definitions. What's the difference?

Epistasis under the classical definition describes only interactions in which one mutant phenotype is masked or suppressed in the presence of the other mutation. The population geneticist's definition includes classical epistasis, but also encompasses 'aggravating' or 'synthetic' interactions - where two mutations together yield a surprisingly deleterious phenotype [3].

OK, you've defined epistasis. But why should I care about it?

Epistasis, in the classical sense, provides a logical framework for inferring biological pathways from biochemical and other experiments, because it suggests that two genes are working within the same pathway and sometimes in what order they act. This makes epistasis analysis a very important tool in functional genomics experiments where pairs of genes are systematically deleted so that any interactions can be detected and interpreted in terms of biological interactions or pathways [4]. Epistasis analysis has already informed our understanding of the components and their order of action in every biological process we can think of.

Every biological process you can think of, maybe, but that doesn't help me. What kind of process are you talking about? And why doesn't non-classical epistasis tell you about pathways too?

All right, let us give you two examples.

First, the yeast genes *BNI1* and *BNR1*, which encode so-called formin proteins involved in the nucleation of actin filaments, have an aggravating genetic interaction (epistasis in the non-classical sense). A mutation in either *BNI1* or *BNR1* causes cell polarity defects, but the yeast remain viable. However, deletion of both *BNI1* and *BNR1* in the same cells causes lethality (that is, they have a so-called synthetic lethal phenotype). The *BNI1* and *BNR1* pair exemplifies an aggravating interaction - and the information to be gained from non-classical epistasis more generally.

By contrast, we can look at an example of classical epistasis from the nematode worm *Caenorhabditis elegans*, in which a well studied genetic pathway controls the fate of 'Pn' cells that differentiate to form the hermaphrodite worm's vulva. These cells undergo three sequential differentiation steps, first into 'Pn.p' cells, then into VPC cells, and finally into vulval cells (Figure 1). Three genes control these steps: *lin-26*, *lin-39* and *let-23*. In *lin-26* mutants you don't get Pn.p cells, while in *lin-39* single mutants you don't get VPC cells and in *let-23* mutants you don't get vulval cells. In *lin-26* + *lin-39* double-mutants you don't get Pn.p cells, so the double mutant looks like the *lin-26* mutant -

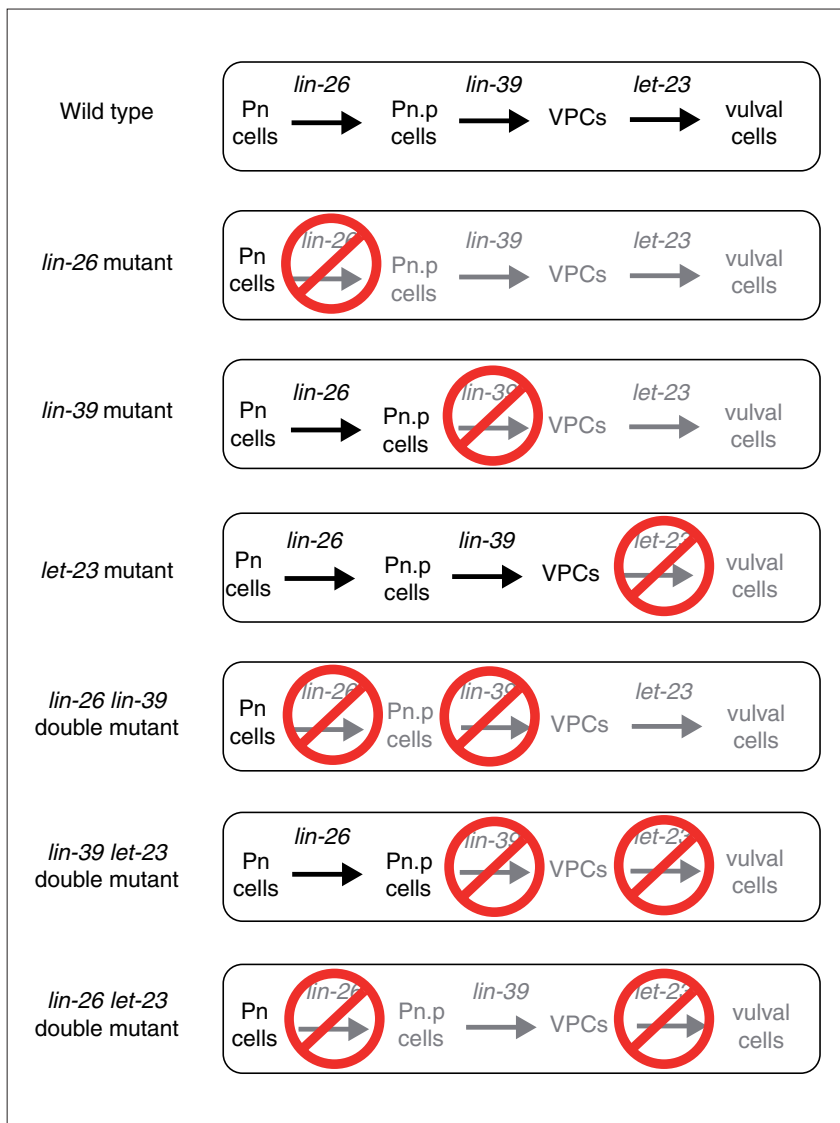


Figure 1
Classical epistasis in the vulval differentiation pathway of *C. elegans*.

that is, the effect of *lin-39* is masked by the effect of *lin-26*, and thus *lin-26* is 'epistatic to', and upstream of, *lin-39*; similarly, in *lin-39* + *let-23* double mutants you don't get VPC cells, so *lin-39* is epistatic to, and upstream of, *let-23*. In a formal sense, this cell fate pathway is similar to a biosynthetic pathway in which the product of one gene's action becomes the substrate for the next gene and so on. In such pathways, the predominating mutation

is always epistatic to the masked or suppressed mutation. The masked or suppressed mutation is said to be 'hypostatic to' the predominating mutation.

So the epistatic gene always acts upstream of or before the hypostatic gene in the pathway?

Not always. This is a good rule of thumb for positive regulatory pathways,

like the one in the example we have just given, in which each step provides the basis for the next, or for biosynthetic pathways where genes encode enzymes that convert a substrate into a product.

If epistatic mutations aren't always upstream, when would an epistatic mutation act downstream?

When the upstream gene product represses the downstream gene product, rather than activating it (or providing a substrate for it). Consider a two-step gene regulatory pathway in which gene X represses gene Y. Let's say that gene Y causes fur to grow on the tip of a heffalump's nose (Figure 2). But of course you know that heffalumps do not have fur growing from the tip of their noses; and this is because gene X represses gene Y. So, a mutation in gene X will result in failure to repress Y and thus the heffalump's nose-tip will be furry. In contrast, a mutation in Y would result in lack of fur on the tip of the nose, since Y is required for fur growth. In the double-mutant, since Y function is abrogated it no longer matters that X isn't there to repress Y, and the nose tip will be beautifully bald (as it should be). In this case, mutations in Y are epistatic to mutations in X, even though Y acts downstream of X.

But how do I know whether I am dealing with a positive-regulatory or biosynthetic pathway, or a negative regulatory pathway, in which the interpretations of epistasis are polar opposites?

The diagnostic sign of a negative regulatory pathway is that mutations at different steps of the pathway result in opposite phenotypes. For this reason, Linda Huang and Paul Sternberg refer to negative regulatory pathways as 'switch regulation pathways' [5]. This is true of our heffalump pathway above, where a mutation in one step gives a hairy nose tip and a mutation in the

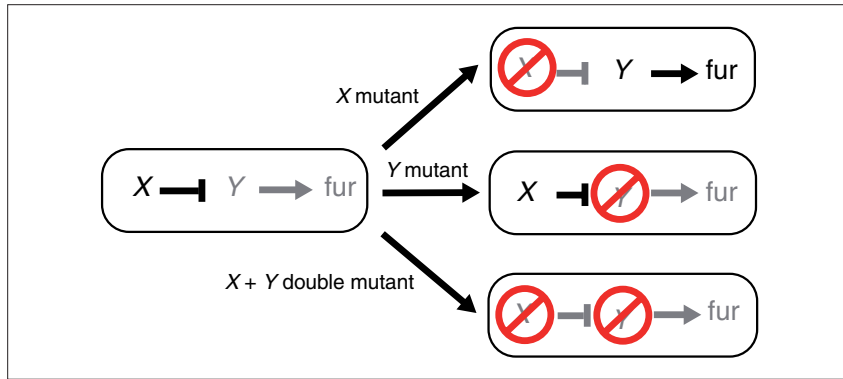


Figure 2
Epistasis in the nose-tip fur of *Heffalumpus*.

which inactivates a membrane protein, TRA, which represses genes that are required for male characters (Figure 3). Mutations that cause loss of function in *her*, the gene encoding HER, cause XO animals to look female, but have no effect on XX animals, because HER is not required for the expression of hermaphrodite characters. In contrast, *tra* loss-of-function mutations cause XX animals to become male instead of hermaphrodite, because TRA is required for the expression of hermaphrodite characters; but XO animals become male just as they should. Double mutants (*tra* + *her*) look like *tra* mutants: XX animals become male. So *tra* is epistatic to *her* and is downstream of *her*, because this is clearly a switch pathway.

next a bald nose tip. A real-life example is sex determination in *C. elegans*, in which there are two sexes,

hermaphrodites, which are XX, and males, which are XO. Maleness is determined by a secreted protein, HER,

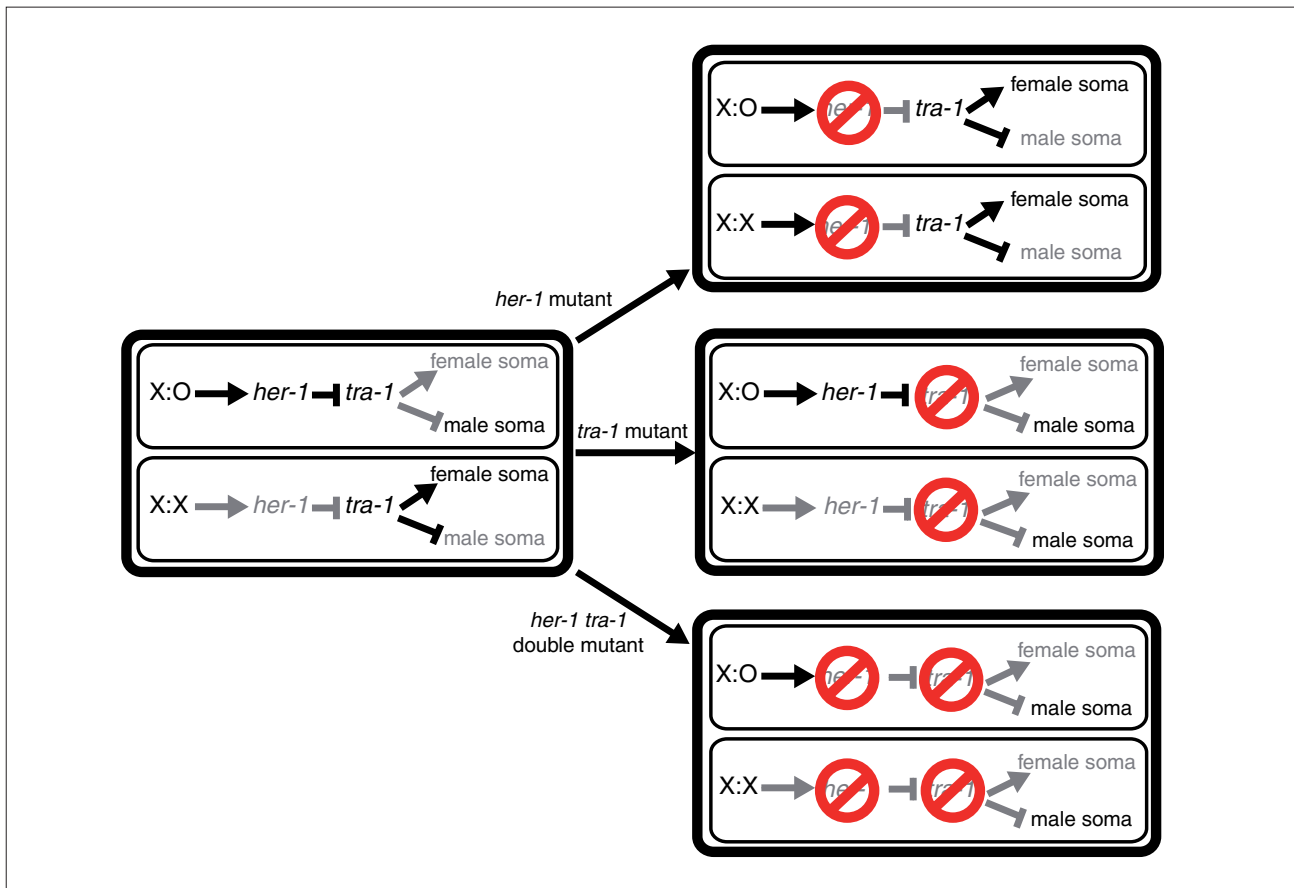
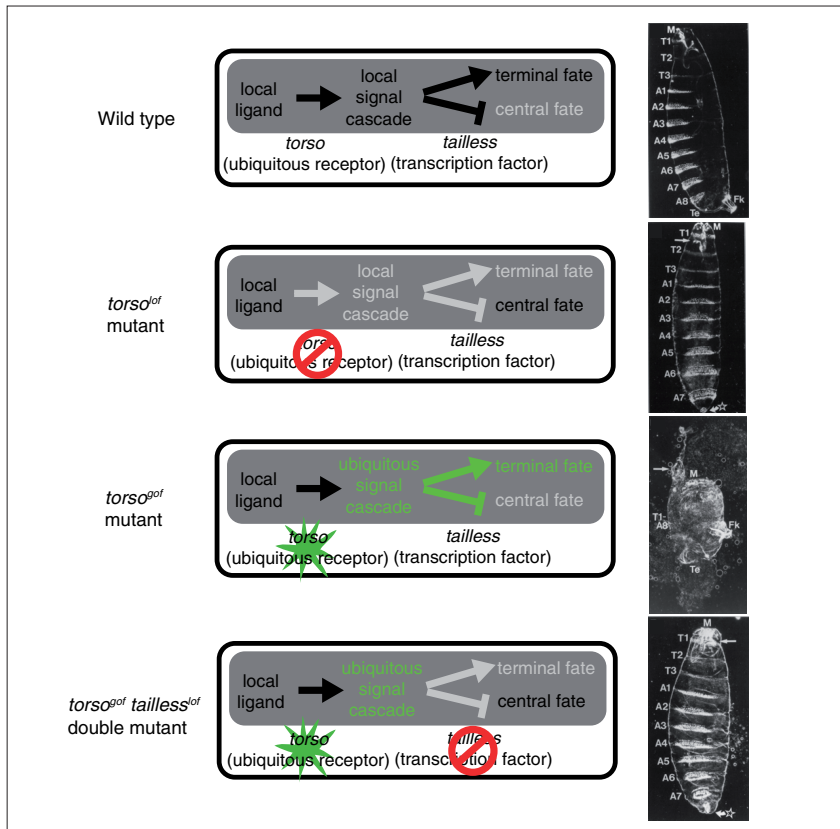


Figure 3
Classical epistasis in the sex determination pathway of *C. elegans*.

**Figure 4**

Epistasis or 'suppression' of a gain-of-function mutation in *Drosophila*. In early *Drosophila* development, the terminal cells differentiate from the central cells in response to signaling through the Torso protein, a receptor tyrosine kinase that is expressed on all the cells of the developing embryo. Torso signaling is confined to the termini through localized release (or processing) of Torso's ligand, which activates the receptor, resulting ultimately in transcription of the *tailless* gene. Tailless is a transcriptional regulator that specifies terminal cell fates and represses central cell fates. In *torso* loss-of-function mutants (*torso^{of}*), all cells develop as central cells. In *torso* gain-of-function mutants (*torso^{of}*), the receptor is constitutively active and all cells develop as terminal cells. In the double mutant, loss of *tailless* function masks the effect of the *torso* gain-of-function mutation and all the cells differentiate as central cells.

Note that not every upstream-downstream relationship exhibits an 'epistatic to' relationship. For example, two mutant genes may yield the same phenotype if, for example, one gene product is required to recruit the other into an active complex. In such cases, we might expect the double mutation to yield the same pathway-disrupting phenotype as either alone. This kind of genetic interaction has been called 'complementary gene action', although some prefer the term 'co-equality' [6].

So how can you learn about pathway order when mutation of either gene yields the same phenotype?

Even if both genes have mutants with the same phenotype, there may be other mutations that enable pathway ordering via epistasis analysis. Specifically, if you can find a mutation that causes a gain of function - for example, by constitutively activating a gene product that normally requires activation. Consider the genes that specify the fates of cells at the termini of

the *Drosophila* embryo so that they are distinct from those in the central region of the embryo. A ligand present only at the termini activates a receptor tyrosine kinase, encoded by the *torso* gene (Figure 4). The activated kinase initiates a signal transduction cascade that ultimately activates transcription of the *tailless* gene in the termini. The *tailless* gene encodes a transcriptional regulator that directs terminal-cell fates and represses central-cell fates in the termini. Thus, loss-of-function mutations in *torso* (*torso^{of}*) and *tailless* (*tailless^{of}*) have very similar phenotypes: the cells at the termini adopt central fates, and classical epistasis is not immediately possible. Epistasis was made possible by the discovery of constitutive gain-of-function mutations in *torso* (*torso^{of}*) in which all cells in the embryo adopt terminal fates [7]. HJ Muller referred to this type of mutation in 1932 as 'hypermorphic' [8]. The *torso^{of} tailless^{of}* double-mutant phenotype was identical to that of *tailless^{of}*, enabling the gene order to be depicted as drawn in Figure 4. Obviously, the constitutive activation of the torso kinase has no effect when the downstream *tailless* gene is inactivated.

On the other hand, mutations that don't cause complete loss of function can be a problem. Let's go back to the nematode sex-determining pathway in which HER negatively regulates TRA. But now assume that while the *tra* mutations are null, the ones in *her* are leaky - or hypomorphic, in the terminology (also devised by HJ Muller in 1932 [8]). The normal function of HER is to turn off TRA. So in a *her* mutant, TRA is turned on. Now in a double mutant in which the *tra* allele is null, you get XX animals becoming male, as described above, and so *tra* is epistatic to *her*. But if the *tra* allele is not null, then in the double mutant the XX animals may still take on some hermaphrodite character together with some male character, so the epistatic relationship would be unclear.

As far as I can see, epistasis analysis works properly only if you already know the pathway functions - so what use is it?

Not at all! Taking the *torso* pathway as an example, the remarkable thing is that the pathway was figured out using genetic experiments before either gene was cloned and found to be in the one case a receptor and in the other a transcription factor. Genetic and molecular experiments complement each other: if only molecular biology were available, there would have been no way of linking the receptor and the transcription factor in regulating the same developmental event; while, if only genetics had been available, then no understanding of the mechanism would have been possible. As another example, the first-known microRNA, *lin-4*, was first shown to be a repressor of its target gene, *lin-14*, based largely on the observation that *lin-14* null mutations cause a phenotype opposite to that of *lin-4(lf)* and are epistatic to *lin-4(lf)* [9].

Do all genes that work together need to have an upstream-downstream relationship?

No. Although some co-equal interactions may correspond to upstream-downstream relationships that may be revealed when the right mutation comes along, many may simply correspond to genes that are working together as a cohesive unit. For example, a systematic genetic analysis of a well studied set of DNA repair genes found nine out of ten co-equal genetic interactions corresponded to protein interactions [6], and these included a 'clique' of co-equal interactions amongst all pairs of the four genes encoding a single complex (the SHU complex).

Now I understand what epistasis is, and how to analyze it, what sort of applications might it have?

As we have already said, there has been a recent wave of information from

functional genomics experiments, including efforts to systematically map genetic interactions. The availability of these data, combined with information on genome variation from next generation sequencing and other techniques, means that we have a remarkable opportunity to apply genetic analysis to reveal components and order of action in biological systems on a global scale. Systematic study of pairwise interactions is now feasible, and for genetically accessible systems such as yeast may even encompass all gene pairs.

What sort of thing can be learned from analysis of systematic interaction data?

One kind of analysis is comparison of genetic interaction profiles. For example, if gene A has 12 synthetic lethal interaction partners, and gene B has synthetic lethal interaction with the same 12 genes, their genetic interaction profiles are entirely overlapping. Indeed, several systematic studies have now clearly shown that clusters of genes with similar profiles often correspond to protein complexes or other biochemical modules, leading to many specific (and subsequently confirmed) biochemical predictions [10-12]. As just one example, *YMR299C* (now called *DYN3*) was predicted on this basis to be part of the dynein-dynactin pathway, which is involved in spindle assembly, nuclear movement and spindle orientation during cell division [8], a prediction later confirmed [13].

In high school I hated logic. Can I still do epistasis analysis?

Maybe. But you may wish to consider alternatives such as a career in politics or, failing that, investment banking.

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