Technical Notes

Basic Concepts of Genetic Analysis
Hammarlund

The goal of these technical notes is to decode for you the obscure terms, concepts, and specialized techniques in your readings. Don't think that these items are what we think is important: usually the exact opposite is true. You are given these notes so that you can quickly and easily get past these aspects of the papers and focus instead on more conceptual/intellectual aspects of the work.

In the xeroxed readings we label technical words or items that need explaining with numbers that refer to the explanatory footnotes on this handout.

Reading #1
Mutations affecting segment number and polarity in *Drosophila*
Christiane Nüsslein-Volhard and Eric Wieschaus

1. **loci**: plural of locus - a place in the genome where a mutation resides. In this case they just use the term "loci" to mean "genes".
2. **metameric**: comprised of segments. In fly larvae, the anatomy of the thorax and abdomen consists of obviously repeating segments.
3. **anlagen**: plural of anlage - group of cells in an embryo from which a given part of the organism develops.
4. **blastoderm**: an early stage in the embryo.
5. **cell lineage restriction between neighboring segments**: i.e. after this stage all the decendents of a given cell always are found within a single segment of the mature animal.
6. **maternal effect mutation**: mutation that prevents a mother from producing normal embryos, usually by disrupting her ability to make normal oocytes.
7. **morphogen gradient**: in 1980 people guessed, on theoretical and some experimental grounds, that a gradient of some diffusable substance (a "morphogen") would be present along the anterior/posterior axis of the developing embryo. Cells at any position in the embryo could thus determine where in the embryo they are by sensing the concentration of the morphogen. As a result of work initiated in this paper people figured out a lot about how cells really know their positions: there do turn out to be morphogen gradients, but there are also additional mechanisms that help cells refine their positional information.
8. **zygotic genome**: the set of genes in the fertilized egg, consisting of some genes from Mom and some from Dad.
9. **metathoracic...mesothoracic**: Anatomical jargon. In the left panel of Figure 2, the segments labeled T1, T2, and T3 are also called the prothoracic, mesothoracic, and metathoracic segments, respectively.
10. **Cuticle, denticles**: The outside of the larva is protected by a tough layer of material called the cuticle that is secreted by the animal's epidermis. The cuticle has rows of hair-like features on each segment called denticles that appear somewhat different in different parts of the body. To make the figures shown the authors used chemicals to dissolve away the entire larva except for the cuticle. They visualize the cuticle in a microscope using lighting conditions that make the denticles appear to glow white. They examine the denticles to determine what has happened to patterning during development of the mutant animals.

11. **deletion and mirror-image duplication**: as described in the Discusssion, it had previously been observed that removing bits from a developing embryo often results in the remaining parts attempting to regenerate by producing mirror-image of themselves. The authors assume that the primary defects in their mutants are deletions of parts of the embryo, and that the mirror-image duplications arise as secondary defects. They focus their analysis entirely on the primary defects (what is deleted) since these reveal the primary functions of the genes they have mutated. They ignore the secondary defects (the mirror-image duplications) since these may have nothing to do with the functions of the mutated genes. This type of logic is fundamental to interpreting any mutant phenotype.
12. ***fused, wingless***, etc.: *Drosophila* genetic nomenclature. *Drosophila* geneticists give each gene a fanciful name that reflects the phenotype seen in animals carrying mutations in the gene. If the mutations described are recessive, lower-case italics are used to write the gene name (e.g. *wingless*); if the mutations are dominant, the gene name starts with a capital letter (e.g. *Ultrabithorax*). The gene names are often referred to by two- or three-letter abbreviations (e.g. *wingless* becomes *wg*; *Ultrabithorax* becomes *Ubx*).

    Confusingly, different nomenclature conventions are used by geneticists studying different organisms, so you will have to deal with completely different systems when you read yeast, *C. elegans*, mouse, or human genetics papers.
13. **zygotic lethals...**: embryos homozygous for these mutations die.
14. **alleles with lower expressivity... leaky allele**: the simplest kind of mutation (a "null" mutation) completely eliminates the function of a gene. In the real world, however, mutations are often isolated that only partially reduce the function of a gene, or that affect the function of a gene in various odd manners. We will discuss this issue in detail in future lectures. An "allele with lower expressivity" refers to a milder mutation that causes all the animals carrying it to be only mildly affected. A "leaky allele" (more often called an allele with low "penetrance") is a different kind of mild mutation: here many of the animals are severely affected, but others "leak" through and are only mildly affected.
15. **Table 1**: Don't worry too much about this table. The legend describes technical details about isolating and mapping the mutations in so little detail that only a *Drosophila* genetics expert could understand them. This *Nature* paper was intended to present only an outline of the results of this work, and intentionally swept all of the technical genetics details like this under the rug. In our second assigned paper, which these authors published a few years later, they present the technical details of their genetic work more completely.

Reading #2: Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*

C. Nüsslein-Volhard, E. Wieschaus, and H. Kluding

1. **Summary**: this contains lots of technical terms. I won't explain them here but instead will go over each as it arises in the body of the paper. At the end you should be able to read the summary and understand it.
2. **5000 genes, 90% mutate to lethality**: An aside: with new information from the genome projects we now know the estimate they had in 1984 is way off: there are a lot more genes (~20,000?) and maybe 1/3 can mutate to lethality. Lots of these genes mutate to give no easily detectable phenotype. Understanding why is a current challenge in genetics.
3. **61 complementation groups**: a complementation group is a set of recessive mutations that fail to complement each other. All the mutations within a complementation group are

usually hits in the same gene. Mutations in different complementation groups are usually in different genes. Thus the 272 mutations seem to identify 61 different genes.

4. **Materials and Methods.** Here is a brief explanation of the main idea of what they are doing:

The authors are looking for mutations that disrupt the patterning of embryos. They assume such mutations will also kill embryos. Therefore, they must design a strategy that allows them to observe and detect such dead, pattern-defective embryos, and yet still be able to recover other living animals carrying the same mutations (in heterozygous form; we assume the lethality will be recessive). They will need these heterozygous carriers of the mutations in order to further study and analyze the mutations.

The trick is that they will produce thousands of inbred families of mutagenized flies. Each family carries different mutation(s), but all members of a particular family carry the same mutation(s). Within a family, some members are mutant homozygotes (allowing families with interesting mutations to be identified by observing these homozygous dead, pattern-defective embryos), and some are heterozygotes (allowing such interesting mutations to be recovered).

Make sure you understand the above strategy. The first six sections of the Materials and Methods and the first page of the Results just describe the technical details of implementing this strategy.

5. **isogenized *cn bw sp* chromosome used for mutagenesis**: In any genetic screen, you induce mutations on some parental genetic background. The mutants will all be compared to this parental strain. Therefore, it is important that all the animals in the parental strain be genetically identical and have a well-defined genotype.

For reasons you will see, in flies it is technically convenient to screen for mutations on just one chromosome at a time (there are only three major chromosomes, so you can do almost the whole genome in three screens). In this paper they are screening for mutations on chromosome II. Therefore, they start off by picking a parental chromosome II to induce mutations on. "Isogenizing" means they did some inbreeding to produce a parental strain in which both copies of chromosome II are identical. The chromosome they are using carries three recessive mutations (*cn*, *bw*, and *sp*) that do not affect embryonic patterning but will make it easy for them to follow this chromosome around and simplify mapping the mutations they find.

cn and *bw* together turn the fly's eyes white (wild-type eyes are red), although individually these mutations only subtly affect eye color

cn = cinnabar: makes fly eyes turn a lighter red

bw = brown: makes fly eyes turn brownish red

sp = speck: black specks on wings

6. **The *DTS91* chromosome**: This chromosome carries a dominant mutation that kills all animals carrying it when they are raised to 29° C, but allows flies to live at lower temperatures; (DTS=dominant temperature sensitive). As they will describe, using this chromosome allows the authors to kill off some undesired members of their inbred families by simply moving the fly vials to a warmer incubator. This trick saved the them an incredible amount of work: without it they would have had to go through each vial and manually remove those flies. They put a bunch of additional recessive muations on the *DTS91* chromosome (*b pr cn sca*) so that they can easily detect "escapers" - any flies carrying the *DTS91* chromosome that failed to die during the 29° incubation.

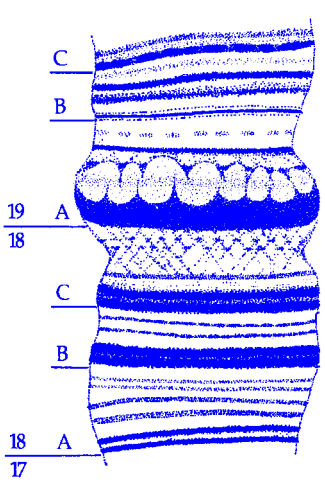
7. **The *CyO* chromosome**: This is a "balancer" chromosome - a very important tool in *Drosophila* genetics. The *CyO* ("Curly of Oster", developed by Mr. Oster) balancer is very sophisticated, and has three important properties:
    A. The defining property of a balancer chromosome is that its DNA is rearranged (for example by inversions of big chunks of the chromosome) in such a way that the balancer chromosome cannot successfully recombine with its homolog. Thus if a chromosome under study is placed heterozygous to a balancer chromosome, these two chromosomes will not exchange genetic information. Therefore the chromosome under study will remain intact and uncorrupted.
    B. The *CyO* chromosome also carries a recessive lethal mutation, so *CyO*/*CyO* flies die.
    C. The *CyO* chromosome also carries a dominant viable mutation that allows one to easily recogize a fly carrying one copy of *CyO*. The *Cy* mutation makes the fly's wings curly. *CyO* also has eye color mutations on it: don't worry too much about descriptions of which combinations of eye pigment mutations produce which eye colors.

    Properties A and B combine to produce a very useful tool. If a recessive lethal mutation *m* under study is placed heterozygous to the *CyO* chromosome, and males and females of this genotype are mated, a "balanced stock" is produced. The *m*/*m* and *CyO*/*CyO* progeny of the cross die, and only more *m*/*CyO* flies are produced. These flies in this stock can continue mate at will with each other and will maintain a population of *m*/*CyO* flies indefinitely without any laborious intervention by the experimentalist (other than tossing some of the flies to a new vial every few weeks to keep them clean and fed). This trick allows the authors to maintain thousands of mutagenized second chromosomes at a time without worrying about losing them. The reason they only screen for mutations on the second chromosome in this paper is that they are only balancing the second chromosome, and thus can only easily maintain mutations on this chromosome. These authors also did separate screens in which looked for mutations on other chromosomes by using balancers for those chromosomes.
7. They describe here other strains used for mapping and complementation - two types of analysis they carry out on the mutations they identify in their screen. I'll give explanations of these below.
8. **Establishment of lethal lines (Figure 1)**. This is the guts of the experiment: it is how they generated thousands of inbred families (called "lines) of animals carrying mutagenized second chromosomes.
9. **ethyl methane sulfonate (EMS)** - is a chemical mutagen. Feeding it to male flies causes mutations to appear in the chromosomes of their sperm. The progeny of these mutagenized males thus inherit from their father a mutagenized *cn bw sp* chromosome. In Figure 1, the fact that this chromosome has been mutagenized is indicated by the asterisk (*).
10. **Single F1 males**: in genetic crosses the generations are kept track of by calling them "F1", "F2", "F3", etc. "F" stands for "filial", an adjective used to describe progeny from a mating. Each F1 male in this cross carries an independent mutagenized chromosome II. Each will be used to generate a separate inbred family carrying that chromosome.
11. **F2 flies of genotype *(cn bw sp)*/CyO***: by cleverly killing all flies carrying the *DTS91* chromosome, the authors have arranged that in each inbred family the only surviving flies are brothers and sisters carrying the same mutagenized chromosome over the *CyO* balancer.

12. **F3 progeny scored for the absence...**: the authors are trying to identify lines that carry a lethal mutation on the second chromosome. Three types of flies are generated in the F3 (in a ratio of 1:2:1, as predicted by Mendelian inheritance):
    A. *CyO/CyO* - these die as larvae and thus never appear in the vial as adult flies.
    B. *CyO/(cn bw sp)**: these have bright red eyes (due to a combination of eye color mutations) and curly wings (due to the dominant mutation on *CyO*).
    C. *(cn bw sp)*/(cn bw sp)*:* these have white eyes (due to being homozygous for *cn* and *bw*) and straight wings (they lack the dominant *CyO* mutation).

    If class C fails to appear in the vial, the authors know that the *(cn bw sp)** chromosome must carry a recessive lethal mutation. They simply go through the vials and pick ones that lack the white-eyed, straight-winged progeny class.
13. **Screening of embryos.** This is their procedure for actually looking at the dead mutant embryos. They let flies from each line lay eggs (using tricks to process 18 lines at a time). They let the eggs that are going to hatch do so and the resulting larvae crawl away, leaving a pile of homozygous mutant dead embryos (note: the *CyO/CyO* animals do hatch and crawl away - they then die later as larvae). The dead embryos are "dechorionated" - i.e. treated with bleach to remove their eggshell. The dead embryos are then treated with chemicals that dissove away everything except the cuticle. The resulting cuticles are then examined in a microscope.
14. **recovery of third-chromosomal mutants**: an extra complication you needn't worry about too much. Sometimes they see interesting mutants popping up that do not appear to be balanced by *CyO* and thus do not appear to be on the second chromosome. When they suspect these mutations might be on chromosome III, they try to balance them with a third chromosome balancer called *TM3*. The few third chromosome mutants they get here just come along as a bonus in this screen. The main way they get a lot more third chromosome mutants (detailed in another paper) is to use *TM3* instead of *CyO* when setting up a separate mutant screen.
15. **putative translocations and semi-dominant maternal effects**. Again, some subtleties you needn't worry too much about. They want to be sure they have isolated simple zygotic recessive lethal mutations. A translocation, one of the more complicated possibilities, is a rare type of mutation in which part of one chromosome breaks off and joins another chromosome. This can change the number of copies present of parts of the genome (an effect called "aneuploidy") and thus cause dominant effects. Semi-dominant maternal effect mutations are another rare possibility that would cause heterozygous mothers to make defective eggs that give rise to defective embryos.
16. **transheterozygote**: an animal carrying one copy each of the two mutations that are being tested for complementation. If the transheterozygote has the recessive mutant phenotype (i.e. has pattern defects and/or dies) then the two mutations fail to complement.
17. **recombination mapping.** The authors want to find the position on the second chromosome of each of the genes they identified. An upcoming lecture is going to be on genetic mapping, so you needn't worry until then about fully understanding this technique. I have nevertheless included an explanation of a couple of the mapping crosses (on separate pages) for those interested - these may seem baffling to you at this point. Notice that they map two different phenotypes. In cross (a) they map the mutation that causes the lethal phenotype. Using cross (b) they map the mutation that causes visible pattern formation defect. The authors hope that for any given line these are one and the same mutation: if both techniques pinpoint the same

position on chromosome II they can be pretty sure that this is the case. Recombination map positions on a chromosome are described by numbers that refer to "map units" along the length of the chromosome (a map unit is a stretch of the chromosome along recombination occurs at a frequency of 1% per meiosis). The second to last column in Table 3 lists the recombination map position of each gene the authors identified.



Schematic drawing of a short stretch of a polytene chromosome

18. **cytological localization**: the authors are using another type of genetic mapping here, called "deficiency mapping", that is much easier to understand than recombination mapping. It allows the authors to refine the position of each of their genes on chromosome II.

    In flies this type of mapping is massively more powerful than in any other organism due to a unique feature of fly anatomy, so you will read about this a lot in fly genetics papers. In the salivary gland of fly larvae each cell has ~2000 copies of the genome, and the multiple copies of each chromosome are bundled together, in register, in a giant cable-like structure called a "polytene" chromosome. These polytene chromosomes are visible in the light microscope, where each chromosome displays a complex and reproducible pattern of dark and light bands. Each really big dark band is assigned a number (1, 2, 3, etc.), lighter bands between the numbered bands get letters (A, B, C, etc.) and even fainter bands between these get numbers again. We can thus refer to any position in the fly genome by these visible features, e.g. "35B2" or "51A1". These are called "cytological" positions, which means (in Latin) that you identify them by "studying cells" in the microscope.

    Over the decades, fly geneticists have generated many fly chromosomes that have suffered small deletions (called "deficiencies"). By just looking in the microsocope at such a chromosome you can see which part of the banding pattern is missing. Table 1 lists all the chromosome II deficiencies the authors use, their cytological positions, and the phenotypes of flies homozygous for each.

    To refine the position of their mutations, the authors test them for complementation to various deficiency chromosomes. A chromosome carrying recessive mutation in a given gene will fail to complement a deficiency chromosome that is deleted for that same gene. This type of test allows the authors to assign many of the complementation groups to very precise cytological position on chromosome II (see the last column of Table 3).

    The precision of this type of mapping is such that these cytological positions often correspond to stretches of less than a few hundred kilobases of DNA. Deficiency mapping is currently used as an important step in cloning genetically defined genes in flies since it allows you to focus on a very small stretch of DNA within which to identify the mutated gene.

19. **establishment of lethal-bearing lines**: see notes 4-12 above which explain the relevant methods sections.
20. ***DTS91* escapers**: see note 6 above.
21. **third chromosome mutations**: see note 14 above.

22. **wierd dominant mutations**: see note 15 above.
23. **Table 2**: this is an interesting analysis and deserves to be thought about carefully. We'll talk about this in the discussion section.
24. **genetic mapping**: see notes 17 and 18 above.
25. **hypomorphic, amorphic, temperature sensitive**,... etc.: These terms are used to describe what kinds of effects mutations have on the functioning of genes. We will discuss this issue in detail in upcoming lectures. "Amorphic" alleles (more often called "null" alleles) completely eliminate the function of a gene. "Hypomorphic" alleles partially inactivate a gene. Temperature sensitive alleles alter a gene such that it can function adequately at one temperature, but is severely impaired for function at another temperature. Temperature sensitive mutations often change a gene such that the protein it encodes is partly destabilized, causing it to unfold at higher temperatures.
26. Expect our class discussion section to emphasize the issues discussed starting in this paragraph and going on to the end of the paper. I hope you will be able to puzzle through most of the "what they did and how they did it" parts of the Results and Methods on your own so that we can spend a significant amount of class time talking about the Discussion section.
27. **antimorphic, neomorphic, synthetic mutations**: These are several classes of odd types of mutations that occur very rarely. The authors are trying to come up with explanations for why a few of their mutations ("single" mutants) complemented all their other mutations. Again, we will discuss these odd kinds of mutations in future lectures so you can hold off worrying about them until then. "Antimorphic" and "neomorphic" mutations cause genes to produce abnormal products that have acquired functions not present in the wild-type products. Other rare mutations cause no phenotype on their own, but do when combined with a mutation in another specific gene - these are "synthetic" mutations.
28. **Can't recover dominant lethals**: the authors are using a lot of jargon to explain the simple point that if a mutation is a dominant lethal, they cannot recover it since the flies will die when they try to balance the mutation over *CyO*. In rare instances genes are "haplo-insufficient lethal" - both copies of the gene must be wild-type for flies to live since one copy cannot make enough product to support viability. "Semi-dominant lethality" means flies carrying one mutant copy of the gene die sometimes, but not always. "Hemizygous" refers to animals that physically have only one copy of a gene, since one of their copies of the chromosome carries a deficiency that deletes the gene.
29. **Mutability of genes by EMS**: the authors are going through every possibility for explaining why some genes might get hit less frequently. Why might one gene be less mutable by the mutagen EMS than another? The simplest example is that very small genes are less likely to be damaged by the mutagen than larger ones because they are simply smaller targets.
30. **Lethal loci and bands**: Ignore this last sentence. The authors are referring to an older, probably incorrect hypothesis that each band visible in the polytene chromosomes corresponds to a gene, combined with another older and incorrect idea that most genes can mutate to lethality. Here is the current thinking: 1) nobody knows what causes the banding pattern in polytene chromosomes or how it relates to where the genes are; 2) interestingly, and contrary to earlier ideas, most genes do not mutate to lethality, or even to give very strong phenotypes. The genetics community is currently trying to come to grips with this second point.