*LECTURE 17*

Other types of mapping:

1. Deletion mapping (pseudodominance in *Drosophila*)

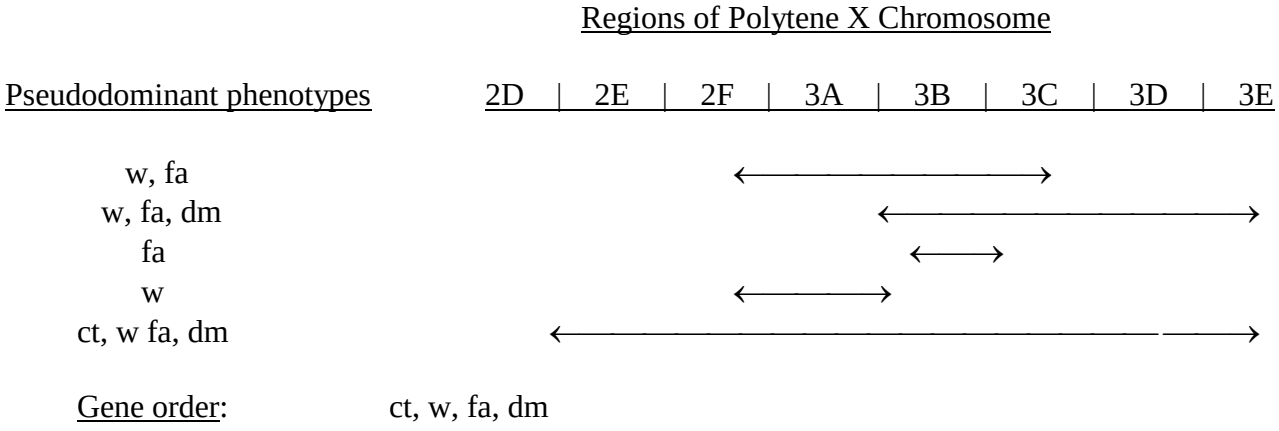
   a) Giant, polytene chromosomes in *Drosophila*: stem from somatic synapsis (homologous pairing) and chromosome duplication in the absence of cell division

   b) Method: Cross multiply-marked recessive to "treated" wild-type homozygote and score $F_1$ for recessive phenotypes. Some will be newly-induced mutations and some will be newly induced deficiencies/deletions. Those that are multiply recessive will most likely be deficiencies (deletions).

      Recover deficiencies by examining polytene chromosomes and identifying deficiency loops. This "uncovers" the genes of interest and allows determination of the physical position(s) of genes.

   Example: $w^+$-; wild-type — $fa^+$- ; wild-type — $dm^+$- ; wild-type — $ct^+$- wild-type
   ww; white eyes — fa fa; facet eyes — dm dm; diminutive — ct ct; cut wings

   Cross: "+" / "+" x w fa dm ct / w fa dm ct ["+" = ZAPPED]




   Gene order: ct, w, fa, dm

   c) Comparisons of physical maps with linkage maps revealed (not unexpectedly) the same gene order. There were notable differences in estimates of "distances" between genes. This illustrated that crossing over does not necessarily occur at the same frequency in all regions of a given chromosome.

   d) Deficiency/deletion mapping is one of the methods for localizing specific genes to specific chromosomes in higher organisms. It's utility is somewhat limited, in that the methods works best in species (largely Dipteran insects) that have polytene chromosomes.

2. Somatic cell fusion/hybridization mapping [widely used nowadays, especially in humans]

a) Fuse somatic cells of two different species (e.g., human x rodent) via polyethylene glycol.

b) Hybrid cells are not produced in large numbers, so one employs a selective medium that only permits hybrid cells to survive.

HAT medium (**h**ypoxanthine-**a**minopterin-**t**hymidine): selects *against* cells that are *not* hybrid

(i) One parental cell line is deficient for the enzyme thymidine kinase ($TK^-$)

(ii) One parental cell line is deficient for the enzyme hypoxanthine phosphoribosyl transferase ($HPRT^-$)

(iii) Neither parental cell line will grow, as aminopterin blocks synthesis of purines and thymidylate and "forces" cells to use a pathway that requires HPRT and TK enzymes

(iv) Hybrid cells survive as long as they retain chromosomes (one from each parent) containing the HPRT and TK enzymes

c) One then develops a panel of biochemical markers for the species (e.g., human) of interest: biochemical markers can include proteins (primary products of genes), DNA, and RNA (of specific genes or not).

d) The biochemical markers are followed for occurrence over several cell generations (remember, these are cells in culture). Chromosomes of one of the two species (usually human, in human x rodent fusion cell lines) are lost in a piecemeal fashion and randomly across different cell lines. The concordant loss of the *same* biochemical markers in different cell lines is an indication that the genes belong to the same *syntenic* group. There are statistical methods to evaluate whether a pattern of concordant loss is significant, but the essence is that markers are lost concordantly in different cell lineages they must be on the same chromosome.

*Synteny or syntenic groups versus linkage groups !!*

e) If a suitable suitable chromosome technology is available where specific chromosomes can be identified unequivocally, one can then localize specific markers to specific chromosomes. This is another (the second discussed) of the methods that can be used to localize specific genes to specific chromosomes.

f) Technical limitations:

(i) only biochemical markers can be "mapped" (no morphological visible mutations)

(ii) the approach is organisms (primarily higher mammals at present) where somatic cell fusion methodology can be used successfully

(iii) to localize “genes” (markers) to a specific chromosome (i.e., not just a syntenic group), a suitable chromosome technology must be available

g) One can employ certain chromosomal rearrangements (*translocations*, primarily) to localize markers to specific chromosomal regions.

h) Currently, mapping studies in a variety of agriculturally important species involves hypervariable markers such as of microsatellites, minisatellites, and AFLPs (randomly generated DNA sequences) to identify QTLs.