How to determine gene order using 3-point crosses.
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Background

Now that genome sequence assembly is nearing completion, order on the genetic map can be predicted for the many identified genes that are positioned on the physical map. Genetic mapping may still be needed, however, not just to complete and confirm the physical map, but also for purposes such as locating mutations that are not yet sequenced and distinguishing different mutant members of gene families.

Two-point crosses that have one marker in common can provide the approximate location of genes within a linkage group, but they may be unreliable for establishing gene order. The marked stocks still in common use in Neurospora are often polymorphic for genes that regulate recombination frequency. Crossover frequencies for the same interval can vary as much as tenfold in crosses of different parentage if parental genotypes differ from cross to cross in genes that regulate meiotic recombination (see for example Fig. 1 in Catcheside and Corcoran 1973). Consequently, if recombination was high in the cross used for one interval and low in that used for another, a tentative order derived from combining recombination frequencies from different 2-point crosses may be incorrect.

Three-point crosses. Three-point crosses provide the simplest and best way to obtain reliable genetic evidence for gene order. The following discussion considers haploid progeny isolated at random rather than as asci. For three linked genes, the key observation is that parental types are most frequent and double crossovers are least frequent among the progeny. This enables gene order to be established unambiguously regardless of the absolute crossover values in the two intervals.

If lengths of the two component intervals are similar, and if numbers of the eight progeny genotypes are tabulated, gene order can be determined by simple inspection. The reasoning goes as follows: Because the genes are linked, the probability of simultaneous crossovers in each of the individual intervals is less than one. Double-crossover progeny can be recognized because they occur with a frequency not greater than the product of crossover frequencies in the two individual intervals. (If crossovers in the two intervals show genetic interference, doubles will be even less frequent than expected from the product of the probabilities for the two singles) The eight progeny genotypes fall into four complementary pairs: parental noncrossovers, single crossovers in the first interval, single crossovers in the second interval, and double crossovers that involve both intervals. Parentals can be recognized as the most frequent pair and doubles as the least frequent. These two classes -- parentals and doubles -- differ from one another at only one marker locus, and this must be in the middle.

If one interval is very short relative to the other, double crossovers and one of the single crossover classes will both be small. Establishing gene order would then require large numbers of progeny if unselected random isolates are used. The problem of disparate interval lengths is best resolved by selecting for crossovers in the short interval and scoring the third, more distant marker only in the selected recombinants. With auxotrophic markers, wild-type recombinants for markers bounding the short interval are readily selected. Critical information on gene order can thus be obtained without the need for complete isolation or recovery of the complementary double auxotroph.

Ordering intragenic sites relative to outside markers. Because gene conversions are frequently accompanied by nearby reciprocal crossovers, the methods used for determining gene order cannot be extended uncritically to determining the order of sites within individual genes. In studies of intragenic recombinantion between heteroalleles, outside markers are scored in the intragenic + + recombinant class. The predominant flanker recombinant has been widely used to infer the order of intragenic sites, as if these sites were mutant alleles at

different loci. However, contradictary results on the order of sites in the *am* gene were obtained when different flankers were used. In an effort to explain the apparent contradiction, Bowring and Catcheside (1999) used very close molecular markers as flankers. Their results confirmed that gene conversions are often accompanied by a nearby reciprocal crossover. They showed that the crosover occurs as a second event, outside the conversion tract, and that the apparent order of sites can be reversed if flanking markers on the two sides are at different distances from the selected gene that underwent intragenic recombination.

Three-point and multiple-point test-crosses were used from the earliest days of mapping in organisms such as Drosophila and maize. Sturtevant and Beadle, in their 1939 textbook, used an example from Drosophila to show how gene order can be determined unambiguously simply by inspection of 3-point data (as in the tables below). Lindegren (1936) used 3-point ascus data to construct the first genetic map in Neurospora. 20 years passed, however, before extensive 3-point data from random isolates were used for mapping in fungi (e.g., Newmeyer 1957, Perkins 1959). \

Crosses with more than three linked markers. Presence of more than three linked markers in the same cross increases information on gene order, but it also increases scoring difficulties and introduces the possibility that ratios will be skewed because multiply mutant progeny or those with certain combinations of markers are poorly viable. Gene order is not immediately obvious when 16 or 32 classes of progeny are produced. In this situation, the easiest way to interpret the data may be to consider the loci three at a time.

Using duplication coverage to determine gene order. When strains with recessive markers in normal sequence are crossed with a duplication-generating insertional or terminal translocation, a marker is known to be included in the translocated segment (i.e., to be 'covered') if the duplication progeny are dominant. Conversely, when duplication progeny are recessive for a liniked marker, the gene is known not to be covered. This holds true no matter how close to a breakpoint the marker may be located.). See *How to use duplication-generating rearrangements in mapping*.

Linkage tests that employ duplication-producing rearrangements can be thought of as 3-point crosses. With insertional rearrangements, if a recessive marker is heterozygous and covered in duplicatgion progeny, it must lie between the two breakpoints in the donor chromosome. If the marker is not covered, determining whether it is left or right of the translocated segment requires a second, noncovered marker in the donor chromosome. With terminal rearrangements, a covered marker must lie between the breakpoint and the terminus of the donor chromosome, while a marker that is not covered must lie between the breakpoint and the centromere. For illustrative examples, see Perkins (1986).

Procedure

Random isolates from a 3-point cross. When markers have been scored in a cross where gene order is not known, genotypes and numbers are conveniently listed using a 3-point data sheet such as that shown in Figure 2 from *How to use genetic methods for detecting linkage*. An example is given here, using numbers from an actual experiment.

Data as originally tabulated using arbitrary gene order			Data retabulated using correct order inferred from progeny numbers		
Genotype a b c	Nos.	Crossover type inferred from progeny numbers	Genotype b a c	Nos.	Crossover type
+ – +	26	Parental	+ – –	19	Parental
– + –	19		– + +	26	
+ + –	1	Doubles	+ + +	14	Singles
– – +	0	Regions 1 & 2	– – –	13	Region 1
+ – –	7	Singles	+ – +	7	Singles
– + +	5	Region 2	– + –	5	Region 2
+ + +	14	Singles	+ + –	1	Doubles
– – –	13	Region 1	– – +	0	Regions 1 & 2
Total	85		Total	85	

Initially, the genes can be listed arbitrarily in any of the three possible orders *a b c*, *b a c*, or *a c b*.. Completion of the tally should reveal which two complementary classes are the most frequent and which two are the least frequent. Two times out of three, the initial tabulation will show the genes in an incorrect order, as shown on the left in the example. The data can then be retabulated showing genes in the correct order and with progeny genotypes correctly identified as parentals, singles, or doubles, as in the table on the right. Organizing the data in this way facilitates calculating crossover frequencies. (Both single and double crossovers must, of course, be used in deriving the value for each interval.) One hundred progeny is usually a reasonable number to isolate initially. If an allele at one locus is lethal or cannot be scored, the scorable member of each complementary class will provide the needed information.

(What is 'left' and what is 'right' in each linkage group is based on convention.)

Gene order from duplication coverage. Enough progeny are needed to distinguish whether alleles at a duplication-linked marker locus show a dominant:recessive ratio of 2:1 or 1:2. See *How to use duplication-generating rearrangements in mapping*. For diagrams and examples, see Perkins (1986). For lists of duplication-generating rearrangements and descriptions of their properties, see Perkins (1997). For map positions of the duplications they produce, see Fig. 2 in Perkins (1977) or the *Duplication Coverage* figure on page 68 in Perkins *et al.* (2001).

References

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