How to use duplication-generating rearrangements in mapping.

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Background

Segmental aneuploids have a long history of usefulness. Deficiencies and duplications have been used for studying gene dosage and dominance (*e. g*., Lindsley *et al.* 1972, Birchler 1983 in Drosophila and maize) and for genetic mapping (*e.g*., Sigurdson *et al.* 1984, Perkins 1986 in Caenorhabditis and Neurospora). Most recently, segmental duplications in Neurospora have provided information on repeat induced point mutation (RIP) (Perkins *et al*. 1997) and meiotic silencing of unpaired DNA (MSUD) (Shiu *et al*. 2001). Deficiencies are most useful in plants and animals, where a haploid segment is displayed against a diploid background, while duplications are more informative in Neurospora, where a diploid segment stands out against a haploid background.

In Neurospora, crosses that are heterozygous for an insertional or terminal rearrangement generate a recombinant class of partial-diploid progeny that are duplicated for a defined chromosome segment. The nontandem duplications produced in this way can be used to provide unambiguous evidence of the genetic map order of rearrangement breakpoints relative to neighboring genes or centromeres. If a linked gene is heterozygous in crosses of *Rearrangement* × *Normal sequence*, the phenotype of duplication progeny will reveal whether the gene is included in the duplicated segment, *i.e*., whether it is right or left of a breakpoint. The sequence is revealed unambiguously regardless of how close the marker is to the breakpoint. Duplication mapping in a haploid organism such as Neurospora is similar in principle to deficiency mapping in diploids, as exemplified by the left-right test devised by H. J. Muller to localize rearrangement breakpoints in Drosophila (See Carlson 1966), or deficiency mapping used by Benzer (1961) to determine the order of mutant sites in phage.

Over 60 duplication-generating rearrangements have been identified in *N. crassa*. These include insertional translocations and transpositions, terminal (quasiterminal) translocations, and terminal pericentric inversions. Breakpoints of the duplicated segments have been mapped with respect to traditional markers in 12 of the 14 chromosome arms (Perkins 1997, Perkins *et al.* 2001). Most of the breakpoints shown on the latest genetic maps were positioned by duplication coverage. With a few exceptions (*e.g*., *T(IIL→IIIR)AR18*, Smith and Glass 1996; *In(VR→VL)2-y am*, Perkins *et al.* 1993), breakpoint mapping has been at low resolution.

Nontandem duplications can also be obtained from intercrosses between two reciprocal translocation or between two inversions that have breakpoints appropriately distributed in the same chromosome arms (Figs. 10-13 and Tables 5, 6 in Perkins 1986).

Meiotically generated Duplication progeny are recognized either by diagnostic marker phenotypes and ratios or by the production of barren perithecia. Perithecia are termed barren if development within them is blocked so that no or few ascospores are produced. Duplications from different rearrangements differ in stability. Barren Duplication strains may, sooner or later, regain fertility. Stability differs for different rearrangements. For information on the stability of

duplications from specific rearrangements, see Perkins (1997). See Perkins (1986) for a detailed account of mapping by duplication coverage, with diagrams and illustrative examples.

Evidence of coverage from allele ratios. The two chromosomes in a duplication-generating translocation can be thought of as *donor* (source of the segment to be duplicated) and *recipient*. If a breakpoint-linked recessive marker on the donor chromosome enters a *Translation* × *Normal* cross from the normal-sequence parent, and if the marker is covered (included in the duplicated segment), one third of the viable progeny will be recessive phenotype and two thirds will be dominant. In contrast, if the marker is not covered, two thirds of the viable progeny will be recessive and one third dominant. Half of the majority class are heterozygous duplications, and these will usually be barren when crossed by a normal-sequence *fluffy* tester (Table 1 in Perkins 1986). Most Duplication strains produce at least a few ascospores when crossed by Normal. Heterozygosity can thus be confirmed by recovering the recessive marker.

If two closely linked recessive markers are present in a normal-sequence donor chromosome, one of which is included in the duplication while the other is not, Duplication progeny will appear to be recombined for the markers (Tables 1, 2, 3 in Perkins 1986). The phenotypically complementary 'recombinant' class is missing because it consists of inviable Deficiency progeny. These were eliminated in the ascospores that did not blacken.

Coverage tests based on marker ratios are, of course, valid only for markers in the donor chromosome. A recessive marker that is closely linked to the breakpoint in the recipient chromosome will show either a 2:1 or a !:2 ratio in progeny from a cross of *Translocation* × *Normal-sequence*, depending on whether the marker entered the cross from the translocation parent or the normal-sequence parent. The majority class will include barren duplications, regardless of marker coupling phase.

Procedure

A strain carrying the duplication-generating rearrangement is crossed to a normal-sequence strain that carries the recessive gene or genes to be tested. About 100 random black ascospores are isolated. The marker is scored and progeny are classed as Rearrangement, Normal, or Barren by crossing them to normal-sequence *fluffy* testers and examining ejected ascospores. (See *How to recognize and diagnose chromosome rearrangements*.)

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