How to recognize and diagnose chromosome rearrangements.

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Background

In most eukaryotes, chromosome rearrangements are recognized cytologically by visibly altered chromosome structure, or genetically by altered linkage relations. The first translocations in Neurospora were detected and verified using these methods (McClintock 1945, Houlahan *et al.* 1949). However, characteristics of Neurospora provide an easier method that depends on ascospore pigmentation, and this has been used to identify most of the Neurospora rearrangements that have been detected since 1950. Viable ascospores are black, but ascospores that contain a recombination-generated deficiency are inviable and fail to darken. (These are referred to as 'hyaline' or 'white'.) Presence of a heterozygous rearrangement is signaled by the production of unpigmented ascospores in characteristic ratios. Preliminary diagnosis of the type of rearrangement can be made by observing patterns of black and white ascospores in individual asci. Diagrams showing the meiotic events that give rise to the various ascus types can be found in Perkins (1974, 1986, 1997) and Perkins and Barry (1977).

Insertional and terminal translocations are difficult to detect using traditional cytological or genetic methods but they are readily revealed when ascus patterns are examined. Insertional and terminal rearrangements generate a class of aneuploid progeny that carry a duplication but no deficiency, and this results in asci with 6 black and 2 white ascospores. This method has been used to identify many duplication-generating rearrangements in Neurospora (see Table 3 in Perkins 1997).

Duplication progeny from insertional and terminal rearrangements are viable, and they can be used to establish whether a breakpoint is left or right of neighboring markers. (Perkins 1986. See *How to use duplication-generating rearrangements in mapping*). Duplications obtained in this way are also valuable tools for varying dosage, determining dominance, and studying chromosome instability, heterokaryon (vegetative) incompatibility, and gene silencing by RIP or MSUD.

Most Neurospora rearrangements are phenotypically normal, or nearly so. However, breakpoints may disrupt a gene, resulting in a mutant phenotype that is inseparable from the rearrangement (see Tables 6 and 7 in Perkins 1977).

Criteria for distinguishing rearrangement-induced ascospore abortion from other causes of pigmentation failure. Rearrangement-generated segmental deficiencies are not the only cause of unpigmented ascospores. White ascospores are also characteristic of Spore killer haplotypes, mutations affecting ascospore color, and .mutations affecting meiosis. Raju (1974) has described how to distinguish between these alternatives.

Fully penetrant crosses heterozygous for Spore killer meiotic drive factors result in 100% of asci with 4 viable black ascospores (B) and 4 inviable, unpigmented 'white' ascospores (W). The same is true for ascospore-color mutants. This distinguishes Spore killers and ascospore color mutations from chromosome rearrangements, which when heterozygous typically produce three major classes of asci, most commonly 8B:0W, 4B:4W, 0B:8W and 8B:0W, 6B:2W, 4B:4W -- see below.) .Mutations that disrupt meiosis or ascus development. are of many types, with variable effects on disjunction and ascospore viability. Their effects on ascospore abortion will generally be highly irregular, with no formation of clearly distinct classes such as are produced by heterozygous translocations. See *How to distinguish a Spore killer from other causes of ascospore abortion*.

Criteria for distinguishing specific rearrangement types.
The following expectations are best understood by referring to diagrams in one of the references cited above.

- *Reciprocal translocations*. Random ascospores are 50% black. 8B:0W and 0B:8W asci are equally frequent. The frequency of 4B:4W asci depends on the distance of breakpoints from centromeres and the frequency of crossing over in the breakpoint-centromere intervals. 2B:6W and 6W:2W asci are absent.

  Shot asci provide information that is not provided when linear asci are observed in opened perithecia. Ascus development is asynchronous in Neurospora, with the result that asci at all stages of development are present in a perithecium, including many immature asci. Unripe ascospores in the immature asci are unpigmented and if the spores were unordered, these asci would be classified as 0B:4W even though they might be of other types. Viable ascospores become fully black only when they are mature, and asci are not shot from the perithecium until they are mature. Shot asci with eight unpigmented ascospores are therefore known with confidence to be *bona fide* 0B:8Ws, but 0B:8W asci can't be distinguished from immature asci of other types in rosettes.

  When one or both breakpoints are close to a centromere, nondisjunction of homologous centromeres ("3:1 segregation") is frequent, and this results in 4B:4W asci. The 4B:4W asci from centromere nondisjuction can be distinguished from the 4B:4W asci that are produced by crossing over because with nondiscjunction all four defective ascospores are located in the same half-ascus (Perkins and Raju 1995).

- *Insertional translocations*. Random ascospores from heterozygous crosses are 75% black because duplication progeny with no deficiency are viable and become pigmented. Insertional and terminal rearrangements are unique among rearrangement types in producing 6B:2W asci, as was first recognized by St. Lawrence (1959) and de Serres (1957). The frequency of 6B:2W asci depends on the distance of breakpoints from centromeres. 4B:0W and 4B:4W asci are equally frequent, while 2B:6W and 0B:8W asci are absent. For a thoroughly analyzed example of an insertional translocation, see Perkins (1972).
- *Terminal translocations*. ('genetically terminal', 'quasiterminal'.) These are similar to insertional translocations in ascospore abortion frequency and in ascus patterns. 6B:2W asci are frequent because the tip breakpoint is necessarily far from the centromere. Rearrangements that involve the nucleolus organizer region at the left end of linkage group V are especially frequent (Perkins *et al.* 1995a).
- *Inversions*. Inviable recombinant products result from crossing over in the inverted segment. The frequency of white ascospores therefore depends on length of the inversion. Consequently, most of the identified inversions have breakpoints located far apart, usually in opposite chromosome arms and often with a breakpoint at one tip. (See Newmeyer and Taylor 1967, Barry and Leslie 1982, Turner and Perkins 1982.) Shorter inversions have no doubt gone undetected because crossing over and the resultant ascospore abortion are infrequent.
- *Intrachromosomal transpositions*. Expectations here are complex, depending on length of the transposed segment, distance between original and new location, whether transposition was to the opposite arm, and whether the inserted segment is inverted relative to its original orientation (see Figure 5 in Perkins *et al.* 1995b).
- *Complex or compound rearrangements*. Typically, fewer than 50% of ascospores are viable. Because genetic analysis is laborious, only a few complex rearrangements have been full analyzed. These

typically have multiple breakpoints involving several chromosomes. See Perkins (1997) for examples.

- *Duplications*. Viable duplication strains are typically barren, but not completely so. Most duplication strains are phenotypically normal or nearly so, but some are abnormal in appearance or growth rate. Duplications heterozygous for alleles at a heterokaryon imcompatibility locus are grossly abnormal, with phenotypes that are characteristically different for each *het* gene (Mylyk 1975. See *How to identify and score genes that confer vegetative incompatibility)*. At least a few ascospores can be obtained from heterozygous crosses of most duplications. As expected, these produce black ascospores, some of which are duplications.
- *Deficiencies*. Meiotic products carrying a segmental deficiency can sometimes be rescued in heterokaryons. When these are crossed, the deficiency segregates 1:1, with four inviable ascospores in each ascus (Barry 1992).

A preliminary diagnosis based only on ascus patterns is sometimes misleading and must be confirmed by further genetic tests. For example, some reciprocal translocations produce one duplication-deficiency product that is able to make black pigment even though it is inviable (commonly found for translocations with a break in the nucleous-organizer arm, VL). Consequently, these produce 6B:2W asci and no 0B:8W asci, simulating an insertional translocation. Their true nature is revealed by mapping breakpoints, by showing that one pair of black ascospores is inviable, and by showing that no barren duplications are produced.

Procedure

Recognition and analysis using crosses with normal-sequence testers.

- *Random ascospores*. Scoring of an isolate as rearrangement or normal sequence can be accomplished by examining the ascospores shot to the wall of a tube or the lid of a plate. The background frequency of nonblack ascospores is seldom more than 5% in isosequential crosses. 75% black ascospores indicates heterozygosity for an insertional or terminal translocation, 50%, a reciprocal translocation, and <50%, a complex rearrangement. *fluffy* testers are recommended for use as protoperithecial parents (Perkins *et al.* 1989, Perkins and Pollard 1989). (See *How to use fluffy testers to determine mating type and in other applications*.)

  Confusion due to bubble-ascus abortion is unlikely and can be completely avoided by using whichever of the RL or OR *fluffy* testers is least closely related to the strain being tested. See *How to minimize bubble ascus formation in crosses for cytology*.

- *Shot asci*. Crosses are made on *fluffy* lawns in petri plates. If progeny from parents in OR genetic background are being tested, *fl* (RL) testers are preferred, to avoid complications from bubble asci (Raju *et al.* 1987). Plates are ready to fertilize after 5 days at 25ºC. Plates are kept inverted throughout incubation and during the collection of asci. Best results are obtained if perithecial beaks are not bent. Since beaks are directed toward a light source, incident light during incubation should come from directly below the inverted plates rather than from one side. Ejection of asci begins about 10 days after fertilization. Plates are placed in the dark for 12 - 24 hours before asci are collected. Ascus shooting is slow when dark-incubated plates are first brought into the light. It is quicker for the next hour or two, then falls off rapidly. Shooting resumes and asci can be collected again after plates have been incubated overnight in the dark.

  Shot asci are obtained as described by Perkins *et al.* (1986) and Perkins (1966b). (See *How to obtain asci as unordered groups of ascospores ejected from the perithecium*.) Groups of eight ascospores are

allowed to shoot onto a 4% water-agar slab placed on a microscope slide that is raised on an underlying stack of slides so that the collecting surface is within 1 mm of the ostioles. Ascospore scatter is a function of distance from the ostiole. Each slab is exposed until numerous well-spaced groups have been shot. This may require only a few seconds or it may take several minutes. Timing is determined by trial and error.

Shot asci are examined at 50× or 60× magnification with illumination from below. For optimal contrast between black and nonblack ascospores, lighting is adjusted by altering lamp intensity or by tilting the substage mirror. Well-separated, unambiguous groups are classified as 8:0, 6.2, 4:4 etc. (B:W). Asci are not isolated if diagnosis of rearrangement type is the main objective. As each ascospore octad is counted and recorded, it is destroyed by being punched into the agar with a needle. Tallies are kept on an appropriate data sheet (Figure 1). About 100 groups are scored for each rearrangement, providing information for determining the type and for estimating centromere-breakpoint distances.

- *Intact asci*. If information is needed in addition to what can be obtained from shot asci, linear asci are examined in rosettes obtained by opening perithecia about 10 days after fertilization. Asci are classified according to numbers of black and white ascospore pairs and the frequencies of first and second-division segregation patterns within the linear ascus. See *How to prepare ascus rosettes for microscopic examination*.

Genetic mapping of rearrangement breakpoints:

- *Linkage group assignment*. Evidence of linkage to traditional genetic markers is required. As a first step, putative translocations are crossed to *alcoy; csp-2*. If two markers in the *alcoy; csp-2* tester show linkage, follow-up testers with markers in normal sequence are available to identify which linkage groups are involved in the translocation being tested (Table 1). (See Perkins 1966a, 1991. See *How to use alcoy for linkage group assignment*.)

  If the four visible markers in the *alcoy; csp-2* tester show no linkage among themselves, the new rearrangement is likely to involve two of the same linkage groups that are already linked in *alcoy*, namely I;II, or IV;V, or III;VI. One option for the next step is to cross the new rearrangement to normal-sequence testers that have markers in these linkage groups (Table 1). Another option (probably more laborious) is to cross the rearrangement to a *multicent* strain in which all seven linkage groups are marked. (See *How to use multiply marked multicent strains for mapping genes and translocation breakpoints*.)

- *Breakpoint location within a linkage group*. Because of duplication coverage, order of the breakpoint or breakpoints in the donor chromosome of insertional or quasiterminal rearrangements can be determined precisely with respect to flanking markers. The rearrangement is crossed by normal sequence strains carrying recessive markers in the donor chromosome. Tests may employ one candidate marker at a time, or multiply marked testers may be used. Coverage of a marker is indicated by a 2 dominant:1 recessive ratio in the progeny, noncoverage by a 1 dominant:2 recessive ratio (if the marker is closely linked) or >1/3:<2/3 ratio (if less closely linked). See Perkins (1986) for examples. See *How to use duplication-generating rearrangements in mapping*.

  As a shade of things to come, Smith and Glass (1996) have used electrophoretically separated chromosome segments to isolate translocation-specific cosmids and to identify, map, and clone breakpoints of an insertional translocation and map them at high resolution.

With reciprocal translocations and other rearrangements that do no produce viable duplications, genetic mapping of breakpoints depends on linkage of the rearrangement to conventional markers. Because this depends on infrequent crossovers in crosses of rearrangement to standard sequence, it is less precise and more laborious than mapping by duplication coverage.

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Table 1. The *alcoy csp* linkage tester and normal-sequence follow-up testers

Genotype	FGSC Numbers	Linkage groups
alcoy; csp-2	3661 (A) 3434 (a)	T(I;II); T(IV;V); T(III;VI); VII
al-1; arg-5	1205 (A) 1206 (a)	I; II
al-1; pe	1203 (A) 1204 (a)	I; II
trp-1; ylo-1	6808 (A) 1208 (a)	III; VI
cot-1; al-3	3612 (A) 3613 (a)	IV; V
cot-1;inl	1243 (A) 1244 (a)	IV; V
al-1; arg-5; csp-2	6664 (A) 6665 (a)	I; II; VII
cot-1; al-3; csp-2	6666 (A) 6667 (a)	IV; V; VII
trp-1; ylo-1; csp-2	6680 (A) 6681 (a)	III; VI; VII
al-1; arg-5; cot-1; inl	1885 (A) 1886 (a)	I; II; IV; V
al-1; arg-5; trp-1; ylo-1	2124 (A) 1888 (a)	I; II; III; VI
trp-1; cot-1; inl; ylo-1	1987 (A) 1988 (a)	III; IV; V; VI
trp-1; cot-1; al-3; ylo-1	4321 (A) 4322 (a)	III; IV; V; VI

TALLY SHEET FOR SHOT ASCI

Testing Strain_____

Linkage Gps._____

Parents:_____ Cross No._____

Date plate inoculated_____

Date fertilized_____

DATE OBS.	8:0	6:2	4:4	2:6	0:8	OTHER
TOTAL NOS.						

Figure 1

How to recognize and diagnose chromosome rearrangements.