

How to use genetic methods for detecting linkage.

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Background

It took 75 years using classical genetic methods to assign the first 1000 loci in *N. crassa* to the seven linkage groups (Perkins *et al.* 2001), but following completion of the draft genome sequence, only months were needed to add another 1000 loci (Borkovich *et al.* 2004; see also http://www.bioinf.leeds.ac.uk/~gen6ar/newgenelist/genes/gene_list.htm). However, despite the speed and facility of determining gene location on the basis of DNA sequence, genetic methods will continue to be needed on occasion. For example, suppressors and mutant genes that are known only by phenotype will need to be mapped. Genetic mapping can help distinguish the members of redundant gene families, and genetic methods will probably continue to be the most convenient way to locate rearrangement breakpoints.

Linkage of mutant or polymorphic markers: Linkage of any two markers is indicated if parental combinations are significantly more frequent than recombinants among progeny from ascospores isolated at random, or if parental ditypes are in excess over nonparental ditypes when asci are analyzed. Random isolates are generally more efficient than tetrads for detecting linkage, both because of the labor involved in isolating asci and separating the ascospores and because each randomly isolated ascospore has originated from a different meiosis (Perkins 1953).

Centromere linkage: When Neurospora first came into use, the ability to use ordered asci for mapping centromeres was a novelty and it was given much prominence. Linearly ordered asci were often isolated laboriously when the same information could have been obtained more economically either with random isolates or with unordered asci. For most current problems, centromere linkage is irrelevant. Ascus analysis to provide knowledge of centromere location can be important, however, in special situations such as in studies of chromosome rearrangements or of genetic factors that effect chromosome behavior in meiosis and ascus development.

Mapping of centromeres relative to other loci depends on the fact that when chromosomes of a bivalent disjoin at the first meiotic division, the two centromeres remain undivided, holding the two chromatids of each half-bivalent together. Only at the second division do centromeres separate as in

mitosis. Tetrad or half-tetrad analysis is thus essential for centromere mapping. Crossing over occurs at the four-chromatid stage. Marker alleles at a locus proximal to the first chiasma in a chromosome arm will segregate with the parental centromeres at the first division while markers that lie distal to a single chiasma will segregate at the second division. In all eight-spored *Neurospora* species, ascus development is programmed to deliver products of the first division to opposite halves of the linear ascus, whereas marker alleles that segregated at the second division will both be present in each half of the linear ascus, producing staggered or symmetric arrangements of the two alleles.

Physically unordered asci can be used to map centromeres in any chromosome of the complement if a recognizable marker is present at any one of the seven centromeres. This is because second-division segregation of a marker that is centromere-distal to a single exchange will result in asci that are tetratypes with respect to the centromere marker. When a *Sk-2* or *Sk-3* Spore killer haplotype is present at the centromere of linkage group III, all ascospores in the distal or the proximal half of each linear ascus are inviable and fail to blacken. First- and second-division segregation of a marker can therefore be determined readily by scoring it in the surviving half ascus (Perkins *et al.* (1986). See *How to use Spore killer for distinguishing 1st- and 2nd-division segregation with unordered asci.*

Genetic evidence that markers are located in nonhomologous chromosomes: When random progeny from two segregating markers show *parental:recombinant* ratios indistinguishable from 1:1, the loci are known to be either in different chromosomes or very far distant from one another in the same chromosome. These alternatives can often be distinguished if tetrads are analyzed, because only a fraction of multiple crossover meioses result in nonparental ditype (NPD) asci, whereas tetratype (T) asci are produced not only by single crossover meioses but also by many of the multiples. Two genes are unlikely to be in the same linkage group if the NPD:T ratio is significantly greater than 1:4, which is the maximum expected for genes far apart in the same chromosome. If genes are in nonhomologous chromosomes, there is no upper limit to the NPD:T ratio. For rationale, see Barratt *et al.* (1954).

Genetic mapping of sequenced fragments: Any sequenced gene or DNA fragment can be mapped genetically by RFLP, using hybridization with a heavily marked set of meiotic progeny from a reference cross as a basis for comparison. (See Nelson and Perkins 2000. See *How to use RFLP for mapping cloned genes.*)

Procedures

Increasing the efficiency of random isolates by using multiply marked testers: Rather than testing an unknown by crossing it to markers in the seven linkage groups one or two at a time, it is more efficient to use multiply marked strains. Testers have been developed that incorporate markers tagging all seven linkage groups. Linkage group assignment of an unmapped marker is most economically accomplished using *alcoy; csp-2* testers, which contain four readily scored visible markers, three of which tag unlinked reciprocal translocations. Each of the translocation-linked markers enables detection of linkage to one or another of the two translocation chromosomes (Perkins 1991). Follow-up testers are available for determining which of the two linkage groups is involved. The four visible *alcoy* markers do not affect viability and are readily scored without transfer to test media. When an unmapped gene is crossed by *alcoy; csp-2* and 100 ascospores are isolated, about two thirds of new mutants show linkage to one or another of the *alcoy* markers. For details, see *How to use alcoy for linkage group assignment*.

Normal sequence strains with centromere linked markers in all seven linkage groups are also available (Perkins 1990). These *multicent* strains have been especially useful for identifying the chromosomes involved in translocations. Scoring is more laborious than with *alcoy; csp-2*, however, and marker allele ratios are not as good. For details, see *How to use multicent linkage testers*.

Linkage between mutant or polymorphic markers

- *Random isolates:* Linkage is indicated by a significant excess of *Parental* over *Recombinant* progeny. Figures 1 and 2 are convenient tally sheets for recording numbers of random ascospore isolates from 2-point and 3-point crosses. It is wise to control against differences in viability or ripening speed by scoring complementary classes of both parental and recombinant progeny..
- *Asci:* Linkage is indicated by an excess of *Parental ditype* over *Nonparental ditype* asci. Ordered asci also provide information on centromere linkage, but this is often irrelevant or of peripheral interest when linkage between genes is being assayed in two-point or multipoint crosses. Random isolates (or unordered asci) will generally be preferred over ordered asci for evaluating linkage between phenotypically distinguishable markers. This is because they provide the required *Parental:Recombinant* (or *PD:NPD*) information with minimal effort.

Mapping rearrangement breakpoints

- *Random isolates:* Scoring of isolates as *Rearrangement*, *Normal sequence*, or *Barren* is accomplished by fertilizing normal-sequence *fluffy* testers in plates or small slants and scoring as described in *How to recognize and diagnose chromosome rearrangements*. See also *How to*

isolate ejected ascospores at random. If ascus patterns indicate a putative translocation (see Perkins 1974), the easiest first option in identifying the linkage group groups of a putative rearrangement is to cross the strain by *alcoy; csp-2* on the chance that linkage will be detected between two *alcoy* markers. If no *alcoy* markers are linked and if ascus patterns in *Rearrangement* × *Normal* indicate that the breakpoints are centromere-linked, the rearrangement is then crossed by a normal-sequence multiply marked *multicent* tester (Perkins 1990. See *How to use multiply marked multicent strains for mapping genes and translocation breakpoints*. If two *multicent* markers show linkage, proximity of breakpoints to markers can then be determined by using ascospore abortion to score for presence of the rearrangement in progeny recombinant for the two linked markers.

- *Asci:* See *How to obtain asci as unordered groups of ascospores ejected from the perithecium.* See *How to isolate ascospores in order from the ascus.*

Ascertaining deviations from a 1:1 ratio: Table 1, taken from Perkins (1994), can be used for determining linkage, either with *Parental:Recombinant* random isolates or with *Parental ditype:Nonparental ditype* tetrads.

References

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Perkins, D. D., A. Radford, and M. S. Sachs. 2001. *The Neurospora Compendium: Chromosomal Loci*. Academic Press. (*alcoy*: Fig.9, p.24; RFLP mapping: Appendix 3, pp. 231-239)

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2-POINT DATA

Parents: _____

Linkage Gp. _____

Cross No. _____

Date Crossed _____

Date Isolated _____

	<u>TYPES</u>	<u>NUMBERS</u>	
Parental	{ _____ _____	{ _____ _____	_____
Recombi- nant	{ _____ _____	{ _____ _____	_____
	Total _____		
	Not Germinated _____		
	Total Isolated _____		
	(Germination _____ %)		

RECOMBINATION

Number/Total	<u>Per cent</u>
_____ / _____	_____ %

ALLELE RATIOS

Locus	No. +	No. -
_____	_____	_____
_____	_____	_____

Figure 1

How to use genetic methods for detecting linkage.

3-POINT DATA

Parents: _____

Linkage Gp. _____

Cross No. _____

Date Crossed _____

Date Isolated _____

	<u>TYPES</u>	<u>NUMBERS</u>	
Parental	{ _____ _____ }	{ _____ _____ }	_____
Single, Region 1	{ _____ _____ }	{ _____ _____ }	_____
Single, Region 2	{ _____ _____ }	{ _____ _____ }	_____
Doubles	{ _____ _____ }	{ _____ _____ }	_____

Total _____

Not Germinated _____

Total Isolated _____

(Germination _____%)

ALLELE RATIOS

Locus No. + No. -

_____	_____	_____
_____	_____	_____
_____	_____	_____

<u>REGION</u>	<u>MARKERS</u>	<u>RECOMBINATION</u>	
		<u>Number/Total</u>	<u>Per cent</u>
1	_____	_____/_____ /	_____%
2	_____	_____/_____ /	_____%

MAP: _____

COINCIDENCE

Regions 1 and 2

Observed Pairs _____

Expected Pairs _____

Obs./Exp. _____

Figure 2

How to use genetic methods for detecting linkage.