

## LINKAGE DETECTION

Suppose we have two genes, A and B, both segregating in our population.

### I. Inspection method for linkage detection

A & B each segregates 3:1:

	<u>Phenotypes in F<sub>2</sub></u>			
	AB	Ab	aB	ab
<u>Recombination value</u>	<u>(a)</u>	<u>(b)</u>	<u>(c)</u>	<u>(d)</u>
0%, Repulsion (R)	2	1	1	0
0%, Coupling (C)	3	0	0	1
50%, C or R (independence)	9	3	3	1

$\frac{ad}{bc}$  quick test:  $\rightarrow$  independence = 1; repulsion linkage < 1; coupling linkage > 1.

### II. Precise methods of detecting linkage

#### A. Separating $\chi^2$ into its components based on goodness of fit:

Used only if mode of inheritance is known.

- Calculate a  $\chi^2$  for goodness of fit to the two gene model with no linkage or segregation distortion.
- Do a  $\chi^2$  goodness of fit to the expected ratio for the first gene/character pair (e.g. "A, a")
- Repeat for second character pair (e.g. "B, b")
- Calculate a  $\chi^2$  for the linkage component

*Example:* F<sub>2</sub> data for *cr* (crinkly dwarf) vs. *ms* (male sterile)  
*cr-ms* x Normal

$$a = 181, b = 33, c = 35, d = 30; N = 279$$

Quick test:  $(181 \times 30)/(33 \times 35) = 4.7$  (coupling linkage as > 1)

We know from the cross that the linkage is coupling.

$\chi^2$  test

Overall segregation Ho = 9:3:3:1	<u>Class</u>	<u>Observed</u>	<u>Expected</u>	<u>(Deviation)<sup>2</sup></u>	<u><math>\chi^2</math></u>
	Cr-Ms-	181	156.9	579.00	3.69
	Cr-msms	33	52.3	372.97	7.13
	crcrMs-	35	52.3	299.29	5.72
	crcrmsms	30	17.4	157.82	<u>9.07</u>
					25.61**

$$\chi^2 \text{ 3df, 0.01} = 11.3$$

So, we clearly have deviation in observed ratio from the expected segregation of 9:3:3:1. Is the deviation due to Cr, Ms, or linkage between Cr and Ms?

	<u>Class</u>	<u>Observed</u>	<u>Expected</u>	<u>(Deviation)<sup>2</sup></u>	<u>P<sup>2</sup> (1df for each)</u>
Cr-cr seg.:	Cr-	214	209.25	22.56	0.108
	crcr	65	69.75	22.56	<u>0.326</u> 0.434,ns
Ms-ms seg.:	Ms-	216	209.25	45.56	0.218
	msms	63	69.75	45.56	<u>0.653</u> 0.871, ns

Linkage segregation:

The difference: Overall seg - Cr - Ms = Linkage

$$25.61 - 0.434 - 0.871 = 24.305^{**}, \chi^2, \text{ 1df, 0.01} = 6.63$$

Test for independence:

- Usable for cases where genetic hypothesis is known or where one isn't.
- Also useful in cases where the data are distorted in some way.
- Basically, the observed *ratio* of one character is used to calculate the expected numbers in each class of the other character.
- Calculation of the  $\chi^2$  based on the observed numbers and these calculated expectations will give an indication of the strength of association or possibly linkage.

Set up a multiple-entry table (contingency table) as follows:

	<u>Cr</u>	<u>cr</u>	<u>marginal totals</u>	<u>Ratio Ms:ms</u>
Ms	a=181	c=35	216	216/279 = 0.774
ms	b=33	d=30	63	63/279 = 0.226
marginal totals	214	65	279	
Ratio Cr:cr	214/279 0.767	65/279 0.233		

Now, if Cr and Ms are independent, then the ratio of Ms:ms will be the same in each class of Cr (i.e., Cr and cr). Similarly, the ratio of Cr:cr will be the same in each class of Ms.

So: Calculate the expected values of a, b, c, and d based on the ratio of Ms:ms

$$\text{Exp. a} = (214) * 0.774 = 165.64 \text{ (alternatively } (216*214)/279)$$

$$\text{Exp. b} = (214) * 0.226 = 48.36$$

$$\text{Exp. c} = (65) * 0.774 = 50.31$$

$$\text{Exp. d} = (65) * 0.226 = 14.69$$

In all cases, the squared deviation is 234.369. Thus, the  $\chi^2$  of each class is

$$a = 1.42$$

$$b = 4.85$$

$$c = 4.66$$

$$d = 15.96$$

for a total  $\chi^2 = 26.89$ , again highly significant at  $p < 0.01$ , indicating that linkage is possibly present.

- Further experimentation is needed to make sure that there is linkage and that the association is not due to other factors.
- This method does not consider the individual genes—because we may not know how they segregate.

Short method for 2 x 2 tables:

$$\chi^2 = \frac{(ad - bc)^2 N}{(a + c)(b + d)(a + b)(c + d)}$$

Denominator terms are marginal totals. Thus, in our example:

$$\chi^2 = \frac{((181 * 30) - (33 * 35))^2 * 279}{214 * 216 * 63 * 65} = 26.94$$

- Compare this value with one in the above.
- This test can be used anytime.
- A multiple entry table can be constructed, whether or not the mode of inheritance is known.