

Perkins, D. D. Preliminary characterization of chromosome rearrangements using shot asci.

Asci that are projected spontaneously from the perithecium provide a rapid means for characterizing chromosome aberrations in structurally heterozygous crosses. Well-separated groups of eight ascospores are collected on agar placed 1 mm below the ostioles of an inverted

cross-plate 10 to 15 days after fertilization, using fluffy A or a (standard sequence) as protoperithecial parent. Asci are scored according to the number of block (non-deficient) and non-block (presumed deficient) spores each group contains. (Blacks are conventionally listed first.)

The following relations are expected a priori:

Type of aberration	Ascus types		Theoretical expectation
	Black: white spores		
a. Reciprocal translocation (assuming both duplication-deficiency products are inviable)	8:0 = 0:8		Originate from non-exchanges. Expected in equal frequencies if centromeres segregate independently."
	4:4		Require crossing over interstitially (i.e., between centromere and a break point). Frequency depends on distances between centromeres and break points in each aberration, and may assume any value from 0 to $> 2/3$ .
	6:2 and 2:6		None expected.
b. Insertional translocation (assuming duplication survives)	8:0 = 4:4		Non-exchanges; expected in equal frequencies.
	6:2		Require interstitial crossing over. Frequency depends on distances from break points to centromeres in each aberration, and may assume any value from 0 to $> 2/3$ .
	0:8 and 2:6		None expected.
(Relations similar to (b) would be predicted for reciprocal translocations where one duplication-deficiency class is viable, i.e. one break is at a dispensable tip.)			
c. Inversion (peri- or paracentric)	8:0		Depend on frequency within the inversion of non-exchanges and 1-strand doubles.
	4:4		Depend on frequency of single exchanges and 3-strand doubles.
	0:8		Depend on frequency of 4-strand doubles; therefore least frequent type.
	6:2 and 2:6		None expected.
(Relations similar to (c) would be predicted for an insertion shift within the same chromosome. A pericentric inversion with one break at a dispensable tip would differ from (c) by having 4:4's above changed to 6:2's, and 0:8's changed to 4:4's)			

The above predictions ignore the consequence of nondisjunction of homologous centromeres ("type-2" segregation), which would contribute 0:8's where it occurred.

The second table shows the frequencies of the various types of asci observed in one series of tests using already well-known rearrangements.

Strain crossed x standard	Ascus type (block: white spores)					Known characteristics of aberration
	8:0	6:2	4:4	2:6	0:8	
T (IV; VI) 45502	<u>48*</u>	5	<u>127</u>	1	<u>44</u>	Reciprocal translocation; both breaks far out.
T (I; VII) S1007	<u>119</u>	10	<u>4</u>	4	<u>68</u>	Reciprocal translocation; both breaks close in.
T (I; III) 4540	<u>12</u>	<u>60</u>	<u>21</u>	0	1	Insertional translocation; one break far out.
T (I; II) 39311	<u>171</u>	40	161	11	<u>9</u>	Insertional translocation; both breaks close in.
In (ILR) H4250	<u>29</u>	<u>110</u>	<u>20</u>	5	-	Pericentric inversion (long), with one break at tip.
In (IR) 1325	8	0	<u>51</u>	4	<u>28</u>	Paracentric inversion (long).
Control (fl A x fl a)	<u>46</u>	2	4	1	1	

\* Types providing critical comparisons are underlined.

Minority, unexpected types may be due to physiological unripeness, nondisjunction, or small undetected aberrations. (5% aborted spores are typically observed in wild-type x wild-type crosses in Neurospora.)

Deviations from theoretical expectations we noted in two cases: T (I;VII)S1007 (the deficit of 0:8 asci may indicate that these break down early or are not ejected efficiently in S1007); and In (IR) 1325 (are the excess 0:8's due to a disruption of meiosis by anaphase bridges? ).

Seventy new aberrations (mostly obtained following light ultraviolet irradiation and filtration enrichment) have been characterized in the same way. Among them about 40 appear to be reciprocal translocations. The remainder include putative insertional translocations (or other rearrangements generating viable duplication-deficiency types), possible inversions, multiple or complex rearrangements, and a number of ambiguous cases.

Ambiguity may result from breakdown of 0:8 asci before shooting, or from addition of 0:8 asci by adjacent-2 segregation. Mir-classification seems likely in cases where three ascospore types are produced, one of which is intermediate (perhaps a late ripening duplication or a deficiency that allows some spore pigmentation). In some cases, such an intermediate type may overlap either normal or defective ascospores in appearance, depending on age or on conditions of observation.

Shot asci provide clear diagnoses of uncomplicated rearrangements of types that have distinct predictions (notably reciprocal and insertional translocations). With other rearrangement types that have less definite predictions, ambiguities are expected, and supplementary information will be needed from genetic and cytological sources, or from opening perithecia to observe ordered asci in situ. To the extent that ratios are distorted by selective disruption of particular ascus types, or from ripening differences, shot asci may be more reliable with some aberrations, and unshot asci with others.

- \* - Department of Biological Sciences,  
Stanford University, Stanford, California. 94305.