Prakash, V. Construction of multiple centromere marker strains in Neurospora crassa.

Departures from Mendelian expectation for the joint segregation of unlinked loci have been recorded in various organisms by a number of workers. Some have suggested that there is a tendency for centromeres of similar ancestral origin to segregate at meiosis to the same pole of the cell, resulting in apparent linkages between unlinked loci. It has been recorded that problems are rather imminently persistent surrounding the detection of preferential or non-preferential segregation of a number of non-homologous chromosomes in organisms where all the products of each meiosis are not recoverable together. Such difficulties, however, largely disappear in cases where all the products of each meiosis are not only kept together within each ascus with an identifiable base and apex but also are arranged in a definite order. In haploid organisms like Neurospora, different centrotypes (differing in the parentage of centromeres) are derivable for anything phenotypically discernable as there are no complications of dominance and recessiveness. This removes several practical implications as are being faced in higher organisms like the mouse, cotton, Drosophila, etc.

Lindegren and some other authors have recently provided further evidence that the segregation of centromeres is not random but that the frequency of paternal and maternal pairs may be significantly above or below 50%. If indeed, segregation behavior appears to be an intrinsic property of the centromeres, such a phenomenon can only be detected by markers linked to centromeres of a known type. The problem of getting a given marker linked to a standard centromere is the problem to which this note is directed. A series of back-crosses is called for, but with random spores the process will be a slow and uncertain one

when the markers are closely linked to the centromere.

If, however, asci are dissected it is possible to select those asci which have second division segregation for the marker and which therefore have a cross-over between the marker and the centromere. The probability of getting the desired association of a given marker and a standard centromere is then increased by 1/2 at each generation and that means $(1/2)^n$ after n generations. In this way the following markers were selected for being close to the centromere of their respective linkage groups (Perkins, D. D., Genetics 44: 1185-1208):

cr (L. G. I.); arg-5 (L. G. II); pro1-1 (L. G. III); col-4 (L. G. IV); lys-1 (L. G. V); ylo (L. G. VI); me-7 (L. G. VII).

To ensure a fair amount of standardization of genetic background, each of these markers was back-crossed separately six successive times with Lindegren wild-type strain (LA). In order to ensure the inclusion of Lindegren wild-type centromeres, each time second division segregants were selected among tetrads.

Since, a) second division segregating asci reveal a cross-over between a marker and a centromere and b) homologous centromeres are known to segregate at the first meiotic division, it is quite possible to transfer a marker (with an unknown centromere) onto a chromatid of its homologous chromosome with a known type of centromere. It should be noted that even within a tetrad showing second division segregation it is impossible to distinguish between "cross-over segregants with the required centromeres" and "non cross-over segregants with the original unknown centromeres". However, after each back-cross, the possibility of selecting a segregant with the required centromere, among such tetrads is 50% (see Fig. 1). The chances of selecting a segregant with Lindegren wild-type centromere after six successive back-crosses therefore becomes 98.44%. Further it is quite conceivable that once a centromere of known ancestry is incorporated, it remains in the strain unless it is outcrossed, and during any subsequent back-crossing no change is envisaged. On the other hand, the chances of selecting a segregant with the wild-type Lindegren centromere, among the random ascospores, during similar successive back-crosses are discouragingly remote. This is because one can never be sure whether the segregant was involved or not in crossing-over between the marker and the centromere, especially when the marker is known to be close to the centromere and its crossing-over percentage with the centromere is low.

After back-crossing and selecting for second division segregants, the markers were assembled to form what is called the "Multiple centromere marker strain" - cr; arg-5; pro1-1; col-4; lys-1; ylo; met-7. This may be a useful strain for linkage data and for studies on parental or non- parental association of centromeres (inter-centromeric segregation studies). ---Department of Botany, University of Malaya, Kuala Lumpur, Malaya.

(The stocks described above are available to other workers. They are being maintained at the Botany School, University of Cambridge, Cambridge, England).

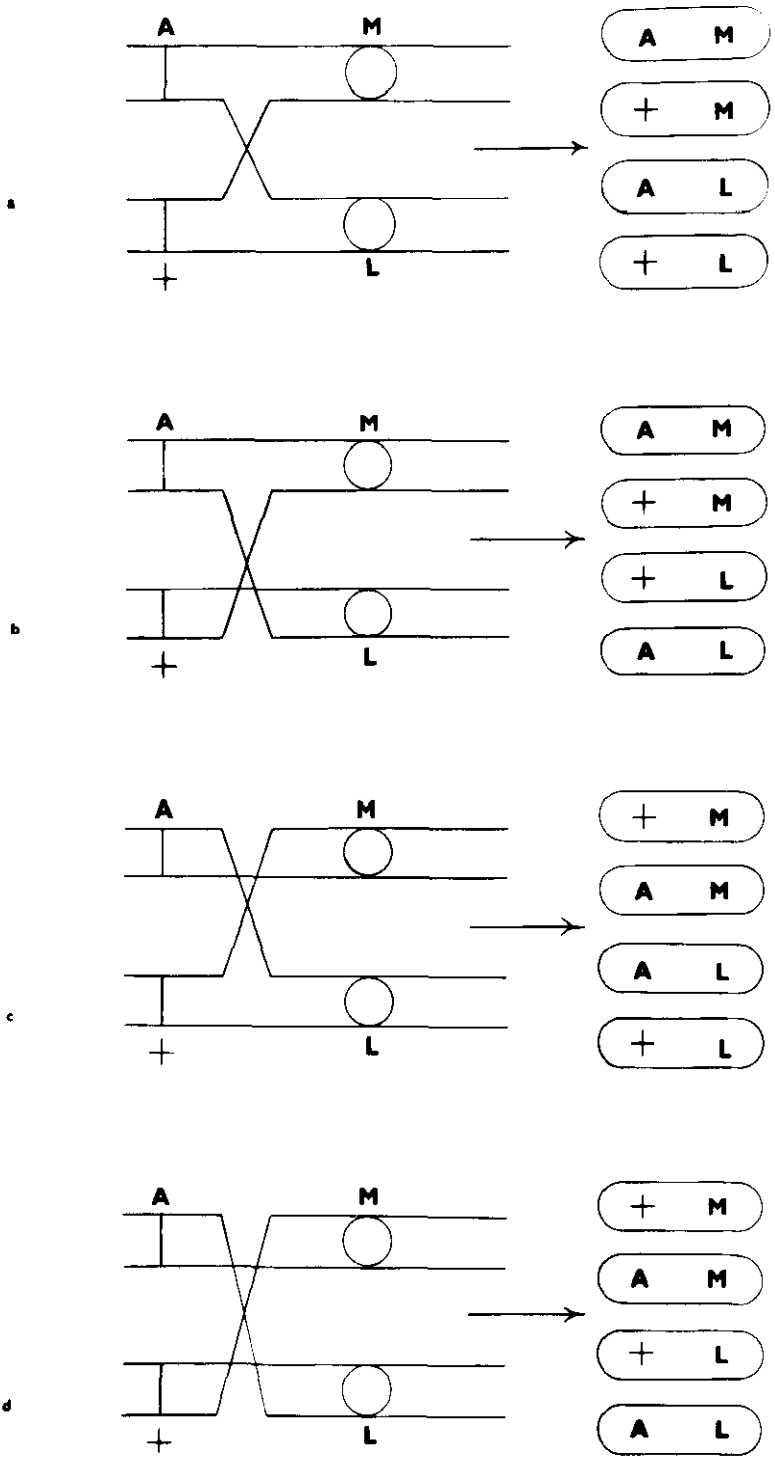

Fig. 1

This shows possible second division segregation configurations of a marker 'A' from its wild-type allele '+' as a result of a single cross-over between the marker and centromere. 'M' denotes a centromere from a strain with mixed ancestry (unknown origin) and 'L' denotes a centromere from Lindegren wild-type strain. Note that there is a 50% chance of selecting a marker segregant with 'L' centromere.