upon the crosses and on the type of reproductive medium used. In certain crosses, they may not be noticed by even about the 22nd day. For purposes of random ascospore analysis, these ejected ascospores are generally isolated without any regard to selection which may be operating at the time of differential discharge exhibited by perithecia. It may be pointed out that certain workers suggest the isolation of ejected ascospores at least eighteen days after perithecium formation, but it is probable that the risk of selection due to 'differential ripening' of asci connected with 'differential discharge' cannot be avoided, even as late as this particular time lag. Perithecia have been found which do not evacuate their full contents altogether by a certain fixed time and some of them appear to do it by degrees. There are others which retain a part of their contents till the time of their disintegration.

Before the method for random ascospore isolation can be described, it may be necessary to have a brief insight into a perithecium. It may also be necessary to explain the outline of the mechanism involved in the 'differential discharge' of ascospores in relationship, particularly, to 'differential maturity' among asci.

A perithecium generally contains a cluster of asci. The cluster exhibits a pattern of its own, as to the arrangement of asci within a perithecium. Some of the asci are situated nearer the perithecial neck than the others and some of them are relatively more involved, as to different degrees of overlapping and crowding within the main body of a perithecium, than the others.

It is often observable that during the course of perithecial ripening, asci show different degrees of maturity. Some asci mature earlier than the others. Even within an ascus, some member ascospores are found at different levels of maturity. Genotype constitution of an ascus, along with other developmental factors, may well be responsible for 'differential maturity'. It may be noted that not only the position of different mature asci varies as to their relative distance from the perithecial neck (the place of discharge) but also the mature asci are found quite indiscriminately attached along with a number of unmatured ones within a perithecium. There appears to be thus a sort of nonlocalization and nonaggregation among the maturing asci.

As a perithecium, on reaching a right stage of maturity, generally starts shedding its ripe ascospores through its protruding neck, it is quite conceivable that those asci near the neck would be more favorably placed as to the shedding of their ascospores than the ones which are distantly situated from the neck. This is why it is not uncommon to find a number of mature asci within a perithecium that has stopped discharging its spores. It may be that, due to their genotypic constitution, certain asci gain such a position within a perithecium that renders them incapable of participating in ejecting their spores, even having reached the right type of maturity. Any sample isolated from the ejected spores may, therefore, preclude any chances of inclusion of genotypes from such asci and this may eventually lead to biased results.

Method: The collection of ascospores is undertaken directly from the fully matured perithecia, instead of the ascospores which are already ejected. This is achieved by running a pilot cross and noting the approximate time for shedding of the ascospores. Fully ripened perithecia are then removed from a cross which is made a day or two later than the pilot one and where no shedding seems to have been involved. A perithecium is judged to be suitable when it sheds spores immediately when placed in a drop of sterile water. All ascospores are isolated irrespective of their color in a microscopic field. ---Department of Botany, University of Malaya, Kuala Lumpur, Malaya.

Prakash, V. Spore isolation in order (Tetrad Dissection).

The following sequence has been found effective during the dissection of asci:

a) Treatment for softening of the perithecial wall
b) Pre and post-treatment washing of perithecia
c) Use of 12% agar blocks
d) Use of sharply pointed tungsten needle for isolation of spores
e) Transferring of ascospores directly to the tubes, instead of first carrying to 1 x 1 x 0.5 mm agar blocks.
Care is taken to exclude as many conidia as possible while removing plump, black perithecia of appropriate ripeness. The perithecia are first washed in five to six changes of sterile distilled water in small separate tubes, so as to remove conidial contamination by vigorous shaking and by draining off the water by means of a pipette. The large difference in density soon separates conidia. For softening the tough perithecial walls and for killing any of the remaining conidia still left over, the perithecia are then transferred to a few drops of fresh 40% chlorox (or 2% sodium hypochlorite) and 1.5% ethyl alcohol solution for three to four minutes. Further careful washing in three to four changes of sterile distilled water is carried out before placing them on a block of 12% agar. The perithecia are squeezed open and ascii are expressed into a drop of sterile water. Ascii containing eight spores in linear order are attached to each other at their bases and extended radially from the point of attachment. The cluster of ascii is pulled to one end of a block of 12% agar and each ascus is separated from the cluster with a pair of sharply pointed needles. Only complete ascii are spread, one by one with a space in between each of the adjacent ascii, on the other end of the block. A drop of water with a pipette facilitates this operation considerably, in carrying the ascii from one end of the block to the other. The block is then allowed to reach a condition of optimum dryness before dissecting ascii. The needles are made by dipping tungsten wire in molten sodium nitrite, heated in a crucible.

A 3 mm thick block (1.5 x 3.5 cm) of 12% agar, instead of the 4% one which is so frequently used, has been found to provide a very convenient, or rather an adequately tough, base for a clean dissection of ascii and without any fear of picking up a stray ascospore. The surface of 12% agar attains the right stage of dryness very easily because of its quick absorption power. In addition, as agar with higher concentration has more water retaining capacity for a particular length of time, a block of 12% agar can last comparatively for a longer time before getting totally dried out, due to evaporation, than the one with 4% agar. Such an agar block affords an added advantage, particularly when a number of ascii are to be dissected at a long stretch of three to five hours. The use of the 4% agar block can almost lead to complete disruption of its surface especially when comparatively immature ascii are dissected, whereas, the latter can be dissected on agar blocks with a higher concentration than 4%, with great ease.

Since it is considered a waste of time in first transferring the ascospores onto 1 x 1 x 0.5 mm agar blocks and then carrying these blocks separately to fresh agar slants in 3 x 3/8 inch test-tubes, the ascospores instead are transferred directly into the tubes containing appropriate medium. There was no evidence that a stray ascospore was picked up at any time.

By employing the above method, it is quite possible, under favorable conditions, to dissect twenty to twenty-five ascii in an hour. ---Department of Botany, University of Malaya, Kuala Lumpur, Malaya.

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.