Frost, L.C. and Greenhill, A.D. Multi-spored Asci in Neurospora crassa.

During the investigation of the effect of temperature on sexual reproduction in Neurospora, the phenomenon of the occurrence of asci with more than eight spores was observed. Such asci will be termed "multi-spored".

The strains used were cr (crisp), re-isolated from strain F945 kindly supplied by Dr. R. W. Barratt, and al-2 (albino), re-isolated from strain 15300 kindly supplied by Professor D. G. Catcheside. Batches of crosses were made by inoculating both strains together on the minimal reproductive medium of Westergaard and Mitchell. Part of each batch was incubated at 25°C. while the rest of the batch was incubated at 30°C. Some of the crosses from each temperature treatment were taken at intervals and the protoperithecia or perithecia fixed, stained and observed cytologically to determine the stage of development reached. Every 24 hours and during meiosis at every 12 hours, some crosses were transferred from incubation at 25° to 30° and some of these also were taken at intervals for cytological observation.

It was found that at 25° protoperithecial growth begins after 24 hours and continues steadily for about a further 48 hours. Development did not take place beyond this stage in crosses incubated continuously at 30°; the protoperithecia darkened but were ultimately devoid of any ascospores. At 25°, however, the protoperithecia expand rapidly in the next 48 hours, Meiosis commenced late on the fifth day and the first wave of asci completed the three nuclear divisions late on the sixth day.

Provided protoperithecial growth had commenced at 25°, crosses transferred from 25° to 30° did undergo meiosis and produce ascospores at 30°. However, a high frequency of aberrant asci was present in all such crosses. As can be seen in Table I, many asci contained more than eight spores. Never more and usually fewer than eight black spores were found in all the multi-spored asci observed. The highest number of spores seen in any one ascus was 22 of which only 3 were black. Asci with fewer than eight spores were also frequent. In addition several asci contained grossly mis-shapen ascospores.

Frequencies of normal and aberrant asci.

Cross* and Treatment ⊦	No. of spores per ascus:- Range	Average	Number of asci:- Normal	8-spored	Multi-spored	Less than 8-spored
1.	8	8.0	23	25	0	0
2.	1-14	7.2	4	7	8	10
3.	1-13	7.0	0	2	10	13
4.	2-14	8.1	1	6	10	9
5.	4-15	9.1	5	8	13	4

* Samples 1-4. Cross: cr, + x +, al-2
Sample 5. Cross: +, + o x cr, al-2 o

⊦ Estimated meioses occurred: (1) all at 25°C. (2) at 25°, during change 25° to 30° and at 30°. (3) during change 25° to 30° and at 30°. (4) all at 30°. (5) at 25°, during change 25° to 30° and at 30°.

Samples 1-5 each based on 25 asci selected at random.

The highest frequency of aberrant asci appeared to occur in those crosses in which meiosis was proceeding in some of the asci when the cultures were transferred from incubation at 25° to 30°. Cytolo-

gical examination of material from such a cross fixed shortly after transfer to 30° revealed, in one particularly favourable preparation, irregular numbers of chromosomes segregating to the poles and the presence of laggards at late anaphase II. Possibly as a result of temperature shock, chromosome segregation was abnormal; the chromosome tended to become scattered and spores were delimited around the scattered chromosomal material.

Single random ascospore isolates were made from the control cross at 25°; a wild-type recombinant, used as protoperithecial parent, and a double mutant, used a conidial parent, were selected for a further batch of crosses. Transfers from 25° to 30° were made when the first wave of ascal meioses commenced. The results were similar to the previous experiments (Table I).

The phenomena reported above are under further investigation. --Genetics Laboratory, Department of Botany, University of Bristol.