Prakash, V. Sexual reproduction of Neurospora in nature.

In nature Neurospora propagates mainly by conidial infection; sexual reproduction leading to the formation of fruiting bodies has never been recorded so far. Some of the reasons for the lack of perithecium formation in the natural state may be as follows: (a) opposite mating type strains may occur at different places and may fail to come into contact; (b) owing to certain "mating incompatibility factors", the strains may show fertility barriers despite contacts between them; (c) favorable physiological conditions may not be provided by the host, e.g., due to the presence of inhibitory substances or departures in pH from the required range for the production of perithecia; (d) both mating type strains on the same host may become "heterocaryotic compatible", instead of initiating hybrid zygote formation leading to perithecium production (in such cases heterocaryons carry within them both the mating types, without undergoing nuclear fusion, so that the heterocaryotic mycelium remains haploid); (e) even when zygotes are formed, they may fail to complete all the developmental stages leading to mature perithecia.

All attempts to get perithecia by dusting or spraying conidia from laboratory stock wild type strains with either mating type A or a onto a host plant infested with Neurospora in its wild surroundings were futile. Perithecia, however, appeared on spraying with a mixture containing two different marked stock strains with opposite mating types onto the wild type growing in nature on the host plant Ficus chamaecarpa (Earth fig). When ordered tetrads and random ascospores were analyzed from such perithecia, an interesting result was obtained, as given in Table I.

Conidial suspensions of the mutant strains were made in water containing the appropriate supplement required for growth. The supplement concentration was several times more than that required for growth of the mutant strains in synthetic medium. In the present case, adenine was added at 2 mg/ml, L-arginine HCl at 3 mg/ml and nicotinamide at 2 mg/ml of water. The suspension contained 1×10^8 conidia/ml. With a sharp scalpel or a needle, multiple incisions were made on the scorched plants (burnt on the surface by fire and still intact in the ground) at close proximity to and on the already growing zones of Neurospora. The mutant strains were then sprayed and the host plants were covered with waterproof paper tents. A good infection was obtained if a small amount of a solution containing the supplements required for growth was periodically added, on the places of multiple incision, once in 48 hrs. From about the fifth day onwards after the spraying, a periodic (once in 72 hrs.) addition of a mixture containing L-asparagine 0.05 mg/ml and L-tyrosine 0.03 mg/ml at the growing points was found to be helpful for the production of perithecia. Perithecia appeared within 23 to 32 days after spraying of the mutant strain mixture.

Class of perithecia	Culture isolates from tetrads and random ascospores	No. of perithecia analysed	Possible origin
A.	All wild type.	5	Wild type* A x Wild type* a
B.	Wild type and mutant type x. (mutant type y was absent)	13	Wild type* A x mutant x, a
C.	Wild type and mutant type y. (mutant type x was absent)	16	Wild type* a x mutant y, A
D.	Both the mutant types x and y.	8	mutant y, A x mutant x, a

* growing in nature on the host plant

Table I

At least one ascus and 15 random ascospores from each perithecium were analyzed. Perithecia were selected at random. The following two mutant strain mixtures were used separately for spraying purposes: (1) ad-5 (71104), a (mutant x) and nic-2 (43002), A (mutant y); and (2) ad-5 (71104), a (mutant x) and nic-3 (30300), A (mutant y). All markers are present on linkage group I.

No perithecia appeared when the experiment was repeated on two other host plants, Saccharum arundinaceum (Elephant grass) and Curculigo recurvata (Weevil-wort). All three of the host plants were fire-scorched and were growing within a distance of 55 to 60 feet from each other.

However, wild type Neurospora collected from the host plant Saccharum arundinaceum produced perithecia on liquid crossing medium containing bacto-peptone 0.25%, sucrose 0.25%, glucose 2.0% and DL-asparagine 0.03% plus the standard salt solution. Crosses were made at 25°C in 150 ml Erlenmeyer flasks on "cottoned" liquid medium (Prakash 1963 NN#4: 25) by adding conidial suspension of the mutant strain mixture (antheridial parent) onto the wild type (protoperithecial parent) which was inoculated 48 hours before crossing. The types of perithecia obtained are shown in Table 2. No perithecia were obtained in the case of the wild type isolated from the host plant Curculigo recurvata, either on the cottoned liquid medium or on conventional slants.

Classes of Perithecia	Crosses performed on:	host plant			cottoned liquid medium		
	Host plant:	Ficus chamaecarpa			Saccharum arundinaceum		
	Mixture No.:	1	2	total	1	2	total
A		3	2	5	4	5	9
B		8	5	13	10	9	19
C		7	9	16	16	9	25
D		5	3	8	0	1	1

Table 2

It is clear from tables 1 and 2 that there are more B and C types of perithecia than A and D types. It may, therefore, be inferred that strains of opposite mating types are present on the host plant. They may be sexually incompatible and grow together without producing perithecia, or else are in different stages of heterocaryosis. It is possible that in the latter case homocaryosis (reconstituting the mating type strains) is first initiated. These reconstituted mating type strains are then amenable to perithecium formation by means of inducible zygosis, i.e., formation of a zygote onsets the formation of another zygote. The legitimate explanation is open to speculation and further experimentation. - - - Department of Botany, University of Malaya, Kuala Lumpur, Malayasia.