FUNGAL GENETICS

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PREFACE

Although the great development of work on fungal genetics, which has occurred during the last few decades, has revealed a great deal which should be of interest to mycologists, most of this work, and this certainly applies to our own, has not been motivated primarily by an interest in fungi for their own sake. Fungi happen to be uniquely suitable and convenient organisms for experimental attacks on a number of important genetic problems. With their rapid life cycles and suitability for fine-structure genetic analysis, they share many of the advantages which have permitted such sensational progress in the fields of bacterial and viral genetics. At the same time, they have chromosomes which, though small, are easily visible and apparently orthodox in behaviour, and their system of sexual reproduction, with its alternation of regular haploid and diploid phases, is similar to that prevailing in higher plants and animals. Thus the fungi offer a good vantage point from which to survey a number of key problems of genetics, such as the mechanisms of genetic recombination and the nature of gene action. It is with these broader implications of fungal genetics that we are principally concerned in this book, although in Chapters 9 and 11 we have attempted to cover some of the more specifically fungal aspects also.

A frequent source of difficulty for beginners in genetics, and even sometimes for experienced geneticists, is the tendency for genetic terms like *gene*, *exchange*, and so on to shift their meanings with the rapid developments of the subject. With the hope of minimizing confusion we have appended a Glossary, which, while it may not command universal agreement, will, at any rate, explain our own usage.

We have been greatly helped by the co-operation of many friends and colleagues. In particular, Drs Raymond Barratt, L. S. Olive, David Perkins, J. R. Raper, Georges Rizet and David Wilkie have been generous in supplying data and manuscripts in advance of publication, and many others, too numerous to mention individually, have helped with advice on points of detail. We are particularly indebted to Dr

Edward Barry, of Yale University, who generously loaned us the negatives from which Fig. 20 was made. We owe a special acknowledgment to our colleague Robin Holliday who has read and given us detailed and constructive criticism of many of the chapters. He has done his best to improve the book, but neither he, nor any of the other friends whose help we have acknowledged, can, of course, bear any responsibility for such faults as still remain.

We are also indebted to Miss Maria Shipton for the care which she has given to typing the manuscript, and to Mr L. S. Clarke who undertook all the photographic work in expert fashion. Permission to reproduce Fig. 14 from *Genetical Research*, Fig. 44 from *Zeitschrift für Vererbungsleure*, Fig. 45 from *Science*, Fig. 46 from the *Proceedings of the Royal Society* and Fig. 47 from *Heredity* is gratefully acknowledged. Finally, we should like to thank Dr K. S. Dodds, Director of this Institute, for his support of our project.

J.R.S.F. P.R.D

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ERRATA

1. The formula in line 7, page 91 should read

$$2/3(1-e^{-3x})$$

2. At the foot of page 104 the following two lines have been omitted:

. . . distinguished from diploids by the size of their conidia. There are also genetic differences. While haploids must, of necessity, show segregation . . .

3. In Fig. 1 (l) an extra small chromosome has been inserted through on error in the drawing.

4. Page 41 line 5: Fig. 41 should read Fig. 40.

CHAPTER 1

THE CHROMOSOME THEORY AS ILLUSTRATED BY THE GENETICS OF *NEUROSPORA*

The chromosome theory forms the main theoretical basis of fungal genetics, as of the genetics of all other groups of organisms. Different groups of fungi have many different kinds of life history, and some of the more important of these will be surveyed in the next Chapter. The fundamentals of the chromosome theory are, however, best illustrated by reference to a single type of organism, and *Neurospora crassa* is certainly the fungal species which has been the most thoroughly studied, both from the genetical and the cytological points of view.

VEGETATIVE STRUCTURE AND REPRODUCTION

Neurospora is a genus belonging to the Ascomycetes, sub-class Pyrenomycetes. Its nutrition is relatively simple, and it grows well on a fully defined medium containing only a simple carbon source (sucrose or glycerol), the vitamin biotin, and inorganic salts. Among its other outstanding advantages are a rapid rate of vegetative growth, a short generation time (the sexual cycle occupies only 10 days under optimal conditions), and self-sterility which permits the making of controlled crosses.

A growing culture of Neurospora consists of branched filaments (hyphae) some 5μ or less in diameter. The growth of a hypha occurs at the tip, although as Zalokar [474] has shown, the bulk of the protoplasm is synthesized some distance behind the tip and transported to it by a vigorous protoplasmic streaming. At 30°C the rate of extension of a hyphal tip may exceed 5 mm an hour. As a hypha grows side branches are formed, each one having the same potentialities for growth as the primary hypha. The whole hyphal system, or mycelium, is subdivided by crosswalls, or septa. The resulting compartments may be called cells, although they are not closely comparable with the cells of higher organisms since each contains numerous nuclei, of the order of a hundred,

rather than a single one. Furthermore, the septa do not form a continuous barrier, but are each pierced by a central pore which allows the passage of streaming protoplasm, including nuclei.

As in all other organisms, the nuclei are self-propagating; that is to say, each nucleus can divide to give two daughter nuclei and this is the only way in which new nuclei can arise. The details of nuclear division (mitosis) in vegetative hyphae are rather hard to make out [13,391] but in essentials the process seems to resemble the more easily studied mitotic divisions in the ascus, to be described below. Briefly, what is seen at mitosis in the ascus [385] is that each nucleus consists of seven-rod-shaped stainable bodies, called chromosomes, and that each chromosome divides longitudinally, one of each pair of daughter chromosomes passing into each daughter nucleus. Thus the self-propagation of the nucleus is due to the individual capacity for self-propagation of its seven constituent chromosomes.

Mycelium growing at the surface of the nutrient medium will send up aerial branches which develop into conidiophores bearing the asexual spores, or conidia, which are the principal means by which the fungus is propagated in nature. The conidiophores are richly branched, and the tips of the branches become subdivided into short segments which round off and are easily detachable as air-borne spores. The conidia, which are bright orange in colour when seen in mass, are quite variable in size and may contain anything from one up to ten or more nuclei [189]. In addition to the more obvious macroconidia, uninucleate microconidia may be formed. Microconidia are only about 1μ in diameter, and are usually far outnumbered by the macroconidia; however special genetic varieties of *Neurospora* will produce microconidia only, and in great abundance [14].

When placed on the surface of an adequate nutrient medium, both kinds of conidia germinate readily to form a hyphal system. Except in the special case of heterocaryosis, where two or more genetically distinct kinds of nuclei are present in the same mycelium, conidia can only propagate the kind of mycelium from which they came, and represent a vegetative, or clonal, form of reproduction.

The sexual cycle and meiosis

A pure strain of *Neurospora* is unable to undergo sexual reproduction; the sexual fruiting bodies (*perithecia*) are only formed when two mycelia, of different mating type, are brought together. Neurospora like other Ascomycetes, has only two mating types which are usually symbolized

by *A* and *a*. There is no morphological difference between *A* and *a* strains, and both can form abundant female reproductive structures, the protoperithecia, when grown on solid (agar) medium of suitable composition [445]. A protoperithecium consists of the ascogonium, which is a coiled multicellular hypha, enclosed in a knot-like aggregation of hyphae. The tip of the ascogonium is extended as a branching system of very slender hyphae, called the trichogyne, which projects beyond the sheathing hyphae into the air. Fertilization occurs when a cell of opposite mating type, which may be a macroconidium, a microconidium, or even a piece of ordinary mycelium, comes into contact with a part of the trichogyne. When such a contact is made, fusion may occur, and one or more nuclei from the fertilizing cell will then migrate down the trichogyne and into the ascogonium [11] (cf Fig. 8).

The details of the nuclear events within the ascogonium after fertilization are not fully understood, but from the work of Colson [69] on the closely related *Neurospora tetrasperma*, as well as from what is known about Ascomycetes generally we can assume that what happens is as follows. No fusion of nuclei of different mating types occurs at this stage. Instead, a pair of nuclei, one from the ascogonium and one from the fertilizing cell, become associated and begin to divide synchronously. The products of these divisions pass, still in pairs of unlike mating type, into numerous *ascogenous hyphae* which now begin to grow out of the ascogonium. While this is happening, the mycelial sheath which enveloped the ascogonium begins to develop as the wall of the perithecium, and becomes heavily impregnated with melanin. The mature perithecium is a flask-shaped structure with a narrow beak-like neck. Occasional perithecia include ascogenous hyphae which have evidently arisen from more than one pair of parental nuclei; such perithecia may well be due to the inclusion of two adjacent ascogonia in a single perithecial wall [290].

The later stages of development of the ascogenous hyphae, and the whole course of development of the asci which arise from them, are well known, thanks to the work of McClintock [261] and Singleton [385] who studied squash preparations of perithecial contents stained with aceto-orcein. Each ascogenous hypha bends to form a hook (or *crozier*) at its tip and the two nuclei of opposite mating type within the crozier divide synchronously (Fig. 1 *a*, *b*). Septa now form to divide the crozier into three cells, the central one, in the curve of the hook, containing two nuclei of unlike mating type. This binucleate cell is the ascus initial, and the two uninucleate cells on either side of it commonly fuse to reconstitute a binucleate cell which can grow on to form a further crozier.

Almost immediately after the formation of an ascus initial the two nuclei within it fuse together (Fig. 1*c*). The chromosomes at this time are in a relatively contracted state, and it can be clearly seen that a nucleus with 14 chromosomes is formed from two nuclei with seven each. The fusion nucleus is described as *diploid*, since it contains a double set of chromosomes. It is, in fact, the only diploid nucleus in the entire life history of the fungus, and its formation is immediately followed by two

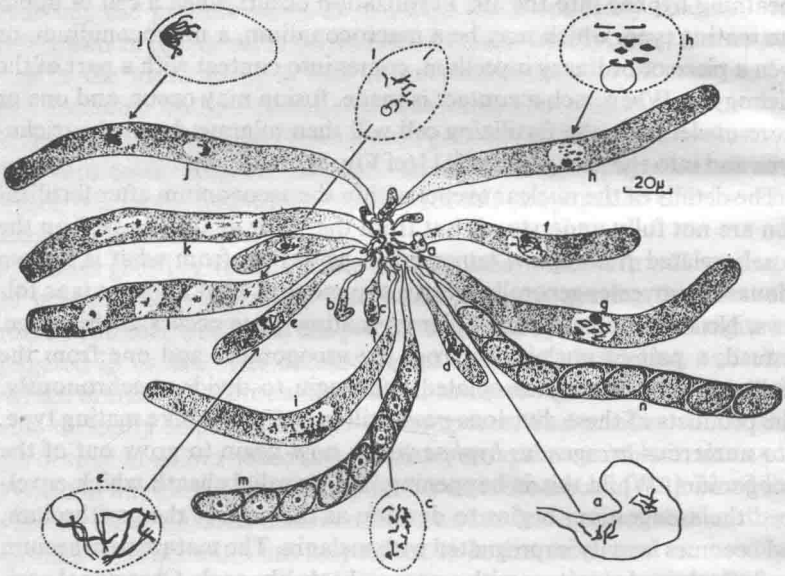

FIG. 1. Stages of ascus development in *Neurospora crassa*. The various asci shown here in the same cluster are based on photographs of several different squash preparations by McClintock & Singleton, using either aceto-carmine or acetic-orcein to stain the chromosomes. The drawing is slightly idealized in the interest of clarity. (*a*) Pro-metaphase of conjugate mitotic divisions in the crozier; (*b*) late mitotic anaphase in the crozier; (*c*) early post-fusion nucleus; (*d*) zygotene to early pachytene; (*e*) mid-pachytene; (*f*) late pachytene; (*g*) diplotene; (*h*) metaphase I; (*i*) anaphase I; (*j*) late telophase I; (*k*) anaphase II; (*l*) metaphase III (mitotic); (*m*) metaphase of mitotic division in the ascospore; (*n*) telophase of ascospore division. Note nucleoli in (*e*)–(*h*) and (*l*) and centrioles at the spindle poles in (*l*).

nuclear divisions of a special kind which between them constitute the process of *meiosis*, and the effect of which is the formation of four nuclei which once again have the *haploid* (i.e. single) chromosome complement. Meiosis occurs as an essential part of the life cycle of all sexually reproducing organisms, and, in its main features, it seems to be the same

process wherever it occurs. The events seen in the *Neurospora* ascus are quite typical of meiosis generally, though not all the stages can be seen in as much detail as is visible in organisms with larger chromosomes. The account which follows is based on the excellent study of Singleton [385].

During meiosis, and especially during the first division, the ascus initial grows rapidly to form an elongated sac, which remains attached to the ascogenous hypha at the base. In the early stages of ascus growth, the chromosomes are seen to undergo lengthwise pairing and subsequently they become progressively more elongated. As a result of this pairing, which occurs during the *zygotene* stages, and which is complete at *pachytene*, it is possible to see that there are seven kinds of chromosome; while different chromosome pairs differ from each other in length, and in the pattern of short deeply staining segments or *chromomeres*, members of a pair are to all appearances identical, with a close point-for-point association all along their lengths (Fig. 1*e*) (Fig. 20).

At the end of pachytene the chromosomes begin to contract again, and there follows a rather ill-defined stage which appears to correspond to the *diplotene* which is clearly visible in some other organisms (Fig. 1*g*). So far as they can be seen, diplotene chromosome pairs in the *Neurospora* ascus, appear to have fallen apart along most of their lengths, remaining joined only at a few points, called *chiasmata*. There is also an indication that the individual chromosomes have divided longitudinally at this stage, but with the two daughter strands still closely associated. Pairs of chromosomes joined by chiasmata are referred to as *bivalents*. Further contraction leads to the *diakinesis* stage, during which the bivalents and their chiasmata are comparatively easy to make out.

Diakinesis is followed by the *metaphase* of the first meiotic division (metaphase I, Fig. 1*h*). The feature of metaphase which distinguishes it sharply from the preceding stages is the appearance in the nucleus of a structure called the *spindle*, consisting of a system of more or less parallel fibres oriented along the long axis of the ascus. The bivalents come to lie in a plane, the spindle equator, which is at right angles to the spindle fibres. Each bivalent lies symmetrically about the equator with one chromosome appearing as if pulled towards each spindle pole. The pull seems to be transmitted through a single point on each chromosome which is called the *centromere* (or sometimes the *spindle fibre attachment*), and which is flanked by two apparently kinetically inert arms.

At *anaphase I* the two chromosomes of each bivalent pull apart towards the two poles of the spindle (Fig. 1*i*), until at *telophase I* the two

resulting groups of seven chromosomes form two daughter nuclei, one in each half of the ascus. The separation of the two nuclei is due principally to the elongation of the spindle, which appears to push the two groups of chromosomes apart. During telophase the chromosomes lose their contraction and during the succeeding *interphase* appear as slender threads which are sometimes visibly double (Fig. 1*j*).

The second division of meiosis is initiated by a *prophase*, during which the chromosomes become contracted once again. This is followed by metaphase II, with the formation of a longitudinally orientated spindle within each nucleus and with the chromosomes coming to lie on the spindle equators. At metaphase II, and also at anaphase I, the chromosomes can sometimes be seen to be divided but with the two halves still held together at least at one point. It is generally accepted, on the basis of analogy with meiosis as seen in higher organisms, that the undivided point on each chromosome is the centromere, though this conclusion could hardly have been reached from a study of the Neurospora ascus alone. Genetic evidence, to be considered below, confirms that the chromosomes must already be divided along most of their length before anaphase I.

At anaphase II each chromosome completes its longitudinal division, and a movement of chromosomes essentially similar to that of anaphase I (with sister centromeres passing to opposite poles) results in the formation of four nuclei arranged in a row (Fig. 1*k*). Since the second division spindles seldom or never overlap, the two nuclei in one half of the ascus derive from the same interphase nucleus. Each nucleus now contains seven undivided chromosomes, one of each kind.

At this point the process of meiosis has been completed. It is most simply regarded as two divisions of the nucleus accompanied by only one division of the chromosomes, the chromosomal division occurring partly during the first nuclear division (division of the chromosome arms) and partly during the second (division of the centromere).

During telophase II the chromosomes become elongated once more, but soon contract again for a third nuclear division. This is a mitotic division, essentially similar in effect to the nuclear divisions which occur in vegetative hyphae. By analogy with higher organisms, although this detail can hardly be seen in *Neurospora*, the chromosomes are believed already to be divided, except at the centromere, before the onset of prophase, and they certainly separate completely into identical daughter chromosomes at the end of metaphase. Anaphase and telophase follow in the usual way. The third division spindles are orientated somewhat

obliquely, but do not overlap (Fig. 1*l*) and so the resulting eight nuclei remain in four adjacent pairs, each pair the product of one mitotic division. Around each nucleus the contents of the ascus become organized to form an ellipsoidal ascospore, which, as it matures, develops a thick ribbed wall, impregnated with melanin. During ascospore development yet another mitotic division occurs (Fig. 1*m*, *n*) so that each ascospore contains two nuclei at maturity.

Although, as we shall see, the chromosomes are of primary importance in genetic theory, they are not the only structures to be seen in preparations of dividing nuclei in the ascus. Each nucleus contains a more or less spherical body, the nucleolus, which hardly stains at all with orcein but is very prominent in preparations stained with carmine. The nucleolus is not self-propagating during meiosis; it tends, in fact, to dwindle in size and vanish as division proceeds, and, if it survives to anaphase, is normally left behind by the chromosome movement. A new nucleolus is formed in each telophase nucleus, apparently always at the tip of the short arm of the second largest chromosome. The nucleolus also differs from the chromosomes in varying greatly in bulk between one stage of ascus development and another; it is particularly well developed during the first division of meiosis, and at pachytene to diakinesis it is far larger and more prominent (in carmine-stained preparations) than the chromosomes themselves (Fig. 1, *e.g.*) The size of the nucleolus is probably connected with the amount of protein synthesis within its sphere of influence; during the first division of meiosis there is, of course, only one nucleolus in the ascus, which is an exceptionally large and rapidly growing cell.

Another interesting feature of ascus development is the presence, during the first post-meiotic division, of prominent centrioles at the poles of the spindles (Fig. 1*l*). The centrioles are triangular plates, commonly seen edge-on, of the same order of size as a chromosome. It is probable that they are present at the spindle poles at all nuclear divisions in *Neurospora*, and other fungi, but they seem to be seldom observed except at mitosis in the ascus; Singleton has suggested that their special prominence at this time indicates that they have a role in ascospore delimitation. Centrioles in animals are known to be self-propagating, and the same appears to be true in Neurospora. The two centrioles present at anaphase are derived from a single centriole observable at the preceding prophase. This capacity for self-propagation might appear to qualify the centriole as a carrier of genetic factors, but no evidence for such a function seems to exist. Like the nucleolus, but unlike the chromosomes, the centrioles stain poorly with orcein, but are readily seen in carmine-stained preparations.

GENETIC ANALYSIS BY ISOLATION OF ASCOSPORES

When ripe, each ascus in turn elongates until its tip reaches the neck of the perithecium, the tip ruptures, and the eight ascospores are discharged violently. Ascospores can be recovered from the side of the culture tube opposite the perithecia, spread on the surface of medium solidified with agar, and induced to germinate, either by heat shock (30 min at 60° is customary) or by treatment with furfural [103]. Heat shock is the most generally useful method of breaking the dormancy, which otherwise persists for long periods. Single spores, either before or after germination, can easily be isolated into individual culture tubes, and the characteristics of the cultures to which they give rise determined. It is, however, more informative in some respects to express the cluster of asci from a perithecium on to an agar surface before the ascospores have been discharged, and to isolate sets of eight spores in order from individual asci. Methods for the dissection and isolation of ascospores from asci have been described by Beadle [19] and by Emerson [109]. If one is content to isolate the eight spores from an ascus without regard to order, one can dispense with ascus dissection. Strickland [405] showed that if the ascospores discharged from the ripe perithecia are collected on an agar surface, a high proportion of them are in groups of eight which seem nearly always to have come from single asci. Separating the members of such groups is very much easier than ascus dissection, and since the ascospores will be riper than undischarged ones they tend to germinate better.

Genetic segregation in the ascus

The parent strains of any Neurospora cross necessarily differ in mating type; of each pair of nuclei fusing at ascus initiation, one is derived from the *A* and one from the *a* strain. When the eight spores from any ascus are germinated individually, and the resulting cultures tested for mating type, four are found to be *A* and four *a*. A second important regularity is that members of a spore pair 1 and 2, 3 and 4, 5 and 6 or 7 and 8 (numbering from the top of the ascus to the base) always give cultures of the same mating type. With these restrictions all possible patterns of mating type distribution within asci occur, though not all with equal frequency. The numbers of the six possible arrangements in 274 asci analysed by Lindegren [244] are shown in Table 1.

In the light of our knowledge of the processes of nuclear fusion and meiosis, this kind of inheritance of mating type finds a fairly obvious explanation, namely that the two mating types are determined by a pair of mutually exclusive genetic factors, or *alleles*, as they may conveniently