
1 Genetics of *Neurospora*

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I. Origins of *Neurospora* Research

The Ascomycete genus *Neurospora* was named when Shear and Dodge (1927) described the discovery of perithecia in cultures of a form previously known only as an imperfect fungus, *Monilia sitophila*. This work described and named three new species, two heterothallic with eight ascospores in each ascus (*N. sitophila* and *N.*

crassa), and the pseudohomothallic, four-spored *N. tetrasperma*. Since then, *Neurospora* has become a model filamentous fungus, a reference point for comparison with other species. Investigations on *Neurospora* have been summarized in a recent book (Davis 2000) and other recent publications (Perkins and Davis 2000; Perkins et al. 2001; Davis and Perkins 2002; Perkins 1992). I refer the reader to these for details of many research programs and comprehensive references.

The initial publication on *Neurospora* and others by Dodge that soon followed described the life cycles. He defined the mating type system, governed by alternative factors *A* and *a*, and rationalized their genetic segregation in relation to cytological features of the meiotic process. The clarity of segregational phenomena – and in particular the pattern of “second-division segregation” that appeared in the earliest studies – quickly rendered *Neurospora* a textbook example of the genetics of a haploid eukaryote.

George Beadle’s first encounter with *Neurospora* was at Cornell University in 1929 or 1930, when Dodge lectured there about the genetics of the fungus. Perhaps more important, he was at California Institute of Technology when Carl Lindegren began his strain development of *N. crassa* and genetic mapping of some mutants he had isolated. The tractable genetics, the simple growth requirements, and the haploid condition of the organism qualified it in his mind as one of the few suitable at the time for the isolation and genetic analysis of nutritional mutants. In 1941, Beadle and Tatum published their groundbreaking paper describing the isolation of three X-ray-induced, auxotrophic mutants (Beadle and Tatum 1941). Their laboratories collected large numbers of such strains that would soon convince most biologists that genes controlled fundamental attributes of living cells, the biochemical reactions of central metabolism.

The initial work compelled Beadle, Tatum, and others who joined the effort to develop the genet-

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ics of the organism further. In particular, many mutants, both biochemical and morphological, were mapped and most linkage groups were identified with particular, cytologically detected chromosomes by the mid-1950s. David Perkins, Mary Mitchell and others brought tetrad dissection, begun by Dodge, to a sophisticated level. The use of heterokaryons to perform tests of allelism or nonallelism of similar biochemical mutants was introduced quite early by the Beadle laboratory. Induction of mutants by ultraviolet light and, later, by chemical mutagens, became routine. Media suitable for plating spores followed the discovery that the sugar sorbose induced colonial growth. Mutant isolation was facilitated by several negative selection techniques.

With these developments, the stage was set for explorations well beyond the gene-enzyme question. Indeed, the entire biology of the organism was open to study, based firmly on standardized strains, media and techniques; easy collection and storage of mutants; rapid genetic analysis by both random-spore and tetrad analysis; and the fortuitous appearance or deliberate isolation of many mutants that announced derangements in biological functions of interest. Stock collections were centralized in the Fungal Genetics Stock Center, meetings of the Neurospora Information Conferences began in 1961, and the Neurospora Newsletter bound the community together early in its history. These are some of the reasons that *Neurospora* research has continued vigorously to this day despite its early eclipse by *E. coli* and *Saccharomyces cerevisiae* as better models for many phenomena first studied in *Neurospora* (Davis and Perkins 2002). *Neurospora* has now found a new role as a model filamentous fungus, where it is a point of reference for many genetic and molecular phenomena in fungi. This role has been greatly reinforced by the appearance of the third release of the genomic sequence in the year 2002.

II. Life Cycle and Morphology

Neurospora spp. are saprophytic organisms, growing on carbohydrate-rich substrates such as wood, bread, and the residue of sugar processing plants. Owing to the fact that its ascospores are activated by heat and by furfural (found in plant remains) and to its rapid growth, it is an early col-

onizer of burnt-over vegetation. Its appearance as a bread mold, before the introduction of preservatives, is also understandable. The asexual phase of the life cycle is simple (Fig. 1): mycelia that originate from sexual or asexual spores grow radially, exhausting the substrate and forming aerial hyphae. These hyphae produce the asexual spores, the *macroconidia* (usually referred to as conidia), through branching, budding at the tips, and segmenting. These spores contain one to four nuclei, and are used widely for induction of mutants, phenotypic testing, and plating. The spores are hydrophobic and are easily dispersed by gentle air movements. In addition, and by a quite different morphological sequence, *microconidia* (once known as spermatia) are also formed. These are generally uninucleate, but in practice they are less useful for genetic work owing to their relatively low viability and the abundance of macroconidia in normal culture conditions.

The sexual phase (Fig. 1) requires the participation of mycelia of different mating type (*A* and *a*), both of which can produce female structures, the *protoperithecia*. These are tightly woven hyphae within which lies an *ascogonial* cell, the female gamete. The male gamete can be any viable cell: a micro- or macroconidium, a hypha, or a germinating ascospore. Matings take place through the agency of a *trichogyne*, an extension of the ascogonial cell through the protoperithecial wall. The growth of the trichogyne is ultimately governed by a pheromone emitted by a conidium or other fertilizing agent of the other mating type. Cell fusion is followed by the transportation of a nucleus from conidium to the ascogonial cell body and a series of many divisions of the haploid nuclei of the two mating types in *ascogenous hyphae* within the enlarging *perithecium*. At the tips of these hyphae, *croziers* form where a conjugate division of two nuclei – always of different mating type – occurs. A cell is walled off as the penultimate cell of the ascogenous hypha, and this cell then elongates as the haploid nuclei fuse. This cell is the *ascus initial*, and meiosis takes place without any prior mitotic division of the diploid nucleus. The ascus initial becomes the *ascus* as the meiotic divisions proceed. Owing to the large number of nuclear pairs in the perithecium, it will ultimately produce, though not synchronously, up to 200 asci. In each ascus, the two meiotic divisions are followed by a final mitotic division. When octads of ascospores in asci are almost mature, the asci take turns at the *ostiole* of the perithecium, a

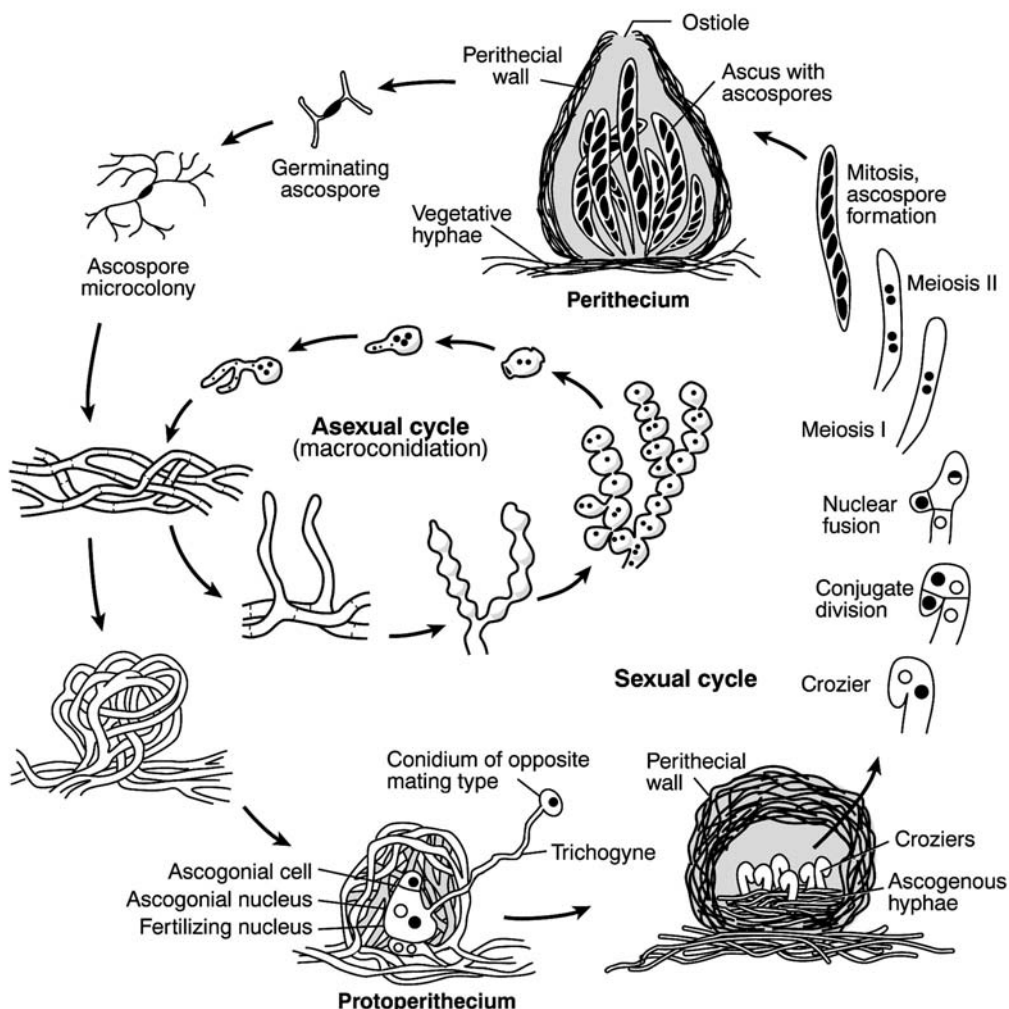


Fig. 1. The asexual cycle (*inner sequence*) and the sexual cycle (*outer sequence*) of *Neurospora crassa*. The asexual cycle proceeds from the formation of macroconidia on aerial hyphae, their dispersal, and their germination to form a new mycelium. Microconidial formation is not shown. The sexual cycle begins with the formation of a protoperithecium containing the ascogonial cell. The ascogonial cell projects a trichogyne to the outside, where it may contact a conidium or other fertilizing element of the opposite mating type. The nucleus of the fertilizing

element travels through the trichogyne to the ascogonial cell. The ascogonial cell develops into ascogenous hyphae, with the haploid nuclei continuing to divide in the process. After growth of ascogenous hyphae and enlargement of the perithecial wall, croziers form prior to fusion of nuclei in the penultimate cells. Ascus formation, including meiosis, culminates in the formation of eight ascospores, which are shot from the ostiole of the perithecium. (Davis 2000; with permission of Oxford University Press, Inc.)

hole from which the ascospores are forcibly shot. The ascospore is a resistant propagule that may remain dormant for some years under proper conditions. The sexual life cycle returns to its starting point upon the germination of the ascospore. Successful germination to a viable state requires water, basic nutrients, and a heat shock (60 °C for 30 min in the laboratory) or an appropriate chemical stimulus (furfural).

III. Growth, Mating, Mutant Selection and Complementation Analysis

Neurospora grows on a minimal medium containing ammonium nitrate, sodium citrate (buffer), potassium phosphate, magnesium sulfate, calcium and sodium chloride, sucrose or glucose, trace elements and a small amount of the vitamin, biotin.

Neurospora extends rapidly over a plate of suitably supplemented agar, followed by increasingly dense growth into the agar. However, addition of the sugar D-sorbose (which inhibits synthesis of the cell wall polymer β -1,3-glucan) and limitation of sucrose or glucose induce intense branching and colonial growth. Conidia may be plated and counted easily after a day's growth on such a modified medium. (Details of methods of *Neurospora* research may be found in Davis and de Serres 1970; Davis 2000.)

A medium designed to induce protoperithecial formation contains much less nitrogen (as potassium nitrate) and carbon source, the latter often represented by filter paper. However, corn meal agar also supports sexual reproduction. One strain is inoculated first on an agar slant and allowed to grow, followed after about 4 days by addition of conidia of the other mating type. Maintained at a temperature no higher than 25°C, perithecia will mature in about 2 weeks, with spores shot to the wall of the culture tube opposite the slant, where they can be collected with a wet bacteriological loop. Suspended in water, they are heat-shocked, plated, and picked from agar under a dissection microscope shortly after germination to individual tubes. The resulting cultures are used for phenotypic testing on suitable plates by stab inoculations of conidia into an agar medium that allows growth into somewhat larger colonies than the plating media used for plate counts and mutant isolation. Selective platings of ascospores are also feasible if, for instance, rare recombinants are sought among cross progeny.

Mutations are induced in conidial populations by applying ultraviolet light to suspensions (in water) of conidia. The conidia are irradiated to about 50% survival, which reduces the viable nuclei per conidium statistically to about one. This eliminates prototrophic nuclei in the same cells as desired mutant nuclei, which will usually be recessive. If a "positive" phenotype is sought, such as resistance to an antibiotic or the reversion of an auxotroph, the conidia are plated in conditions selective for such variants. Any growing colonies may be isolated and purified by serial conidial isolation or crossing. In general, new mutants of any kind are crossed to a strain of the opposite mating type and the desired phenotype is isolated from ascospores, which are definitively homokaryotic. This not only yields pure strains, but may remove additional cryptic genetic variation induced by a mutagen. Many chemicals such as methylmethane

sulfonate and nitrous acid may also be used for mutagenesis (Davis and de Serres 1970).

Two negative selection methods provide means of enriching auxotrophic or conditional mutants such as temperature-sensitive strains. One is to allow the mutagenized population to grow in liquid minimal medium (or medium restrictive to the desired mutant type) on a rotary shaker, with filtration through cheesecloth every 4–6 h. The effect is to remove growing (nonmutant) cells, owing to their filamentous character. The final population of conidia, after 2 days or so, is plated on a sorbose-based medium permissive of the mutants sought. The colonies are picked to individual tubes and tested to distinguish mutants from the remaining prototrophic or wild-type strains. A second method of negative selection is to use, as starting material, a nonrevertible inositol-less (*inl*) strain. Here, growth on media lacking inositol will lead to the death of cells not blocked in germination and early growth, since an unsatisfied inositol requirement during growth is soon lethal. After some time of incubation, the population of cells is overlaid or plated in conditions permissive for growth, including addition of inositol.

Many programs in biochemical or molecular genetics seek mutations of only one phenotypic class. Such mutations include those imposing a requirement for a single amino acid or of the much more general temperature-sensitive, non-supplementable class. The many mutants obtained by enrichment techniques cannot initially be distinguished by their phenotype, but they may be sorted out by *complementation tests*. The multinucleate (coenocytic) character of *Neurospora* hyphae permits the formation of *heterokaryons*, in which nuclei of different genetic constitution coexist and contribute to the phenotype of the mycelium. If, for instance, mutations imposing an arginine requirement are isolated, conidia from individual cultures of the new mutants may be introduced in all pairwise combinations into small lots of liquid minimal medium and incubated. The fusions between the two cell types will lead to heterokaryon formation, but only combinations with nuclei having mutations in different genes will grow on minimal medium (or, as we say, complement) well. This is because what is lacking in one nucleus is provided by the other and vice versa: two separate dominance relationships allow the mycelium to appear normal. Nuclei bearing allelic mutations, however, cannot complement and will not support growth on minimal medium. Com-

plementation tests, therefore, allow one to group allelic mutants and use only one of them for the first steps of physiological analysis. One should be aware, however that only recessive mutations can be successfully tested in this way, and only in conditions restrictive to their (homokaryotic) growth. In addition, heterokaryon formation may be blocked by incompatibility factors in mutants isolated in different genetic background, and certainly in strains of different mating type.

The techniques described above have yielded a huge number of nutritional, morphological, and conditional mutants of *N. crassa*, all of which can be assigned to separate genes. These cover virtually all attributes for which viable or conditional-lethal mutants might be sought, and have provided *Neurospora* biologists the means for studying a wealth of biological processes. Some of the more genetically oriented areas of investigation are described at the end of this chapter. Review of most other areas of *Neurospora* research may be found in Davis (2000) and Perkins et al. (2001).

IV. Formal Genetics

A. Meiosis

Neurospora spp. have seven chromosomes. The meiotic process in *N. crassa* and *N. sitophila* is orthodox, but the asci display the process especially clearly. Before the fusion of the haploid cells in ascogenous hyphae, the chromosomes have already undergone their premeiotic division. When formed, the diploid nucleus in the ascus initial is already at the leptotene stage of prophase I of meiosis. Pairing of chromosomes in zygotene sees the formation of a normal synaptonemal complex in bivalents, fully formed in pachytene. During pachytene, crossing over and gene conversion events take place, followed by partial dissociation of bivalents and entry into metaphase I. The two meiotic divisions (Fig. 2) apportion the four strands of each bivalent to the four meiotic products. In the heterothallic species, the disposition of the spindles of the first and second divisions involve no overlaps, so that each half of the ascus contains the products of one first-division nucleus. The four meiotic products divide by mitosis immediately after the last meiotic division, yielding an octad (a tetrad of spore pairs). The nuclear events make it possible to detect segrega-

tion of alleles (*A* and *a*) at the first division as the pattern *AAAAaaaa*. Second-division segregation, reflecting exchange of particular chromatid parts during pachytene, is signified by patterns *AAaaAAaa* or *AAAAaaAA*, in which both alleles are found in both halves of the ascus (see gene *B/b* in Fig. 2). Unless one has markers linked to the centromere, and which perforce segregate at the first division, ascus dissection, retaining the order of the spores as they occur in the ascus, is the only method of revealing first- and second-division segregation. *N. tetrasperma* follows a somewhat different pattern of divisions, as described hereafter.

B. Segregation

In random-spore analysis, ascospores are collected from many asci, heat-shocked to activate them, and plated. This method has the virtue of ease as well as the benefit of killing all contaminating vegetative cells and conidia. The cultures emerging from ascospore colonies are expected to segregate in a statistical 1:1 phenotypic ratio in the case of a single gene difference between the parents, as described above.

C. Independent Assortment

If the parents of a cross differ in two distinct traits as in the cross $AB \times ab$, and if the genes are on different types of chromosomes, random-spore analysis will yield a statistical 1 *Ab* : 1 *aB* : 1 *AB* : 1 *ab* ratio. This ratio signifies *independent assortment*. However, individual asci will fall into two or three classes (Fig. 2). In the case above, they may have the genotypes *ABABABabababab*, in which only parental genotypes appear, a “tetrad” called “*parental ditype*” (PD). An equally frequent category is expected if the genes assort independently; this is the *nonparental ditype* (NPD), with the genotypes *AbAbAbAbaBaBaBaB*. The equality of PD and NPD tetrads not only signifies the independent behavior of the two bivalents in question, but assures that all four genotypes will be found in equal numbers. Finally, if one of the genes has undergone second-division segregation (and the other has not), a *tetratype* (T) ascus will be seen, with the pattern *AbAbABABababaBaB*. Tetratype tetrads arise through an exchange of chromosome