Fungal Genetics Reports

Volume 20 Article 2

Neurospora crassa and genetics

D. G. Catcheside
Flinders University

Recommended Citation

Catcheside, D. G. (1973) "Neurospora crassa and genetics," *Fungal Genetics Reports*: Vol. 20, Article 2.
https://doi.org/10.4148/1941-4765.1810

Neurospora crassa and genetics

Abstract

Neurospora crassa and genetics

Neurospora crassa and Genetics

David G. Catcheside

Genetics is concerned with the mechanism of heredity. Its distinctive tools are segregation and recombination, just as chemistry and physics are the distinctive tools of biochemistry and biophysics. The study of Neurospora has contributed spectacularly to general genetics in the analysis of recombination, as have some other fungi, notably yeasts, Sordaria, Ascobolus and Aspergillus.

The special advantages of Neurospora led Beadle and Tatum (1941) to choose it for their pioneer search for nutritional mutants to be used as tools in biochemical genetics. The striking success led Tatum (1946) to search for nutritional mutants in bacteria and so directly to the stocks which permitted the first unambiguous demonstration of sexuality in bacteria and to the study of genetic processes in these organisms.

Recombination occurs between paired homologous chromosomes, normally at meiosis. Bridges (1916) demonstrated that crossing over took place after chromosome replication. He showed that XXY Drosophila melanogaster, whose X chromosomes were genetically marked, would yield some XXY progeny whose two X chromosomes were in part alike and in another part unlike. This was the first proof of crossing over at the four stranded stage of meiosis. The evidence was greatly extended by Anderson (1925) using attached -X flies. Further substantial advance was dependent upon analysis of an organism in which all products, presumably four, of one meiosis could be recovered together.

Certainly, Pascher (1918) did this for a cross of two species of the haploid unicellular alga Chlamydomonas, isolating a number of zygotes. Meiosis occurs in these on germination and four motile cells are produced. Parental characters always segregated two and two, two being like one parent and two like the other. Following two pairs of contrasting characters, the tetrads observed were (a) two like one parent and two like the other parent (parental ditype), (b) two of one recombinant combination and two of the other (non parental ditype) or (c) all four different with each cell of the tetrad having one of the four possible combinations of the characters (tetratype). The occurrence of tetratype tetrads showed that segregation could occur at either division of meiosis and was consistent with crossing over at the four stranded stage, but was not a proof of this hypothesis.

Neurospora took over at this stage, but it was not until 1933 that Lindegren found linked factors in N. crassa and showed by tetrad analysis that crossing over occurs at the four stranded stage of meiosis and involves only two of the four strands at any one place. N. crassa is a haploid fungus existing as two mating types. Each is potentially bisexual, if not genetically defective for one or other type of sex organ, but fertilisation occurs only from congress of the two mating types. Wilcox (1928) showed in N. sitophila, a closely allied species, that the four haploid nuclei resulting from meiosis are arranged linearly within the elongated ascus so that the two second division spindles do not overlap. A subsequent mitosis multiplies each nucleus and, again, the spindles do not overlap. Walls are formed around each nucleus and a linear row of eight elliptical spores are formed. Counting the spores 1 to 8, from apex to base of the ascus, the plane of the first division of meiosis lies between spores 4 and 5, while the planes of the second division lie between spores 2 and 3 and spores 6 and 7. Wilcox and later Dodge (1929) made ordered dissections of asci and showed that the genetic factors determining mating type segregated sometimes at the first and sometimes at the second division. Lindegren, who obtained his foundation stocks from Dodge, showed that the frequency of second division segregation was characteristic for each genetic factor, ranging from nearly zero to a maximum of about 67%. Furthermore, linked factors were correlated in the ways in which they segregated. Lindegren's hypothesis was that crossing over occurred at the four stranded stage, involved only two of the four chromatids at any given point and that the parental centromeres of the pair of chromosomes segregated at the first division of meiosis. To a first approximation, the frequency of second division segregation is twice the cross over value, as defined by Sturtevant (1913).

Further advance in the analysis of recombination had to await the further development and the use of the powerful selective tools provided by nutritional mutants, whose growth was therefore conditional. The task of mapping the newly found genes began at once and progressed steadily. In the meantime, McClintock (1945) showed that, in capable hands, the meiotic cytology of Neurospora crassa could be studied. The haploid number was shown to be the same, seven, as the number of linkage groups and some of the chromosomes could be correlated with some of the linkage groups by the use of translocations. This enterprise was continued by Singleton and developed very highly by Perkins and Newmeyer.

Genetics was for long dominated by Morgan's definition of a gene as an indivisible entity, a unit of inheritance. His other definitions of the gene as units of mutation and of function were long subservient. During the sixth decade of this

century, attitudes underwent a revolution mainly due to close examination of interactions between allelic genes. The results are summed in the terms cistron, muton and recon introduced by Benzer (1957). The possession of nutritional mutants in Neurospora crassa and other micro-organisms allowed much more pentrating methods than previously. For example, the physiological test of complementation in heterocaryons to define groups of allelic genes led to the discovery that genes may be grouped either by the absence of complementation between any pair of mutants or by the absence of complementation between pairs of a large fraction of the mutants and of these with all the others in the group, a few of the latter complementing in pairs.

When crosses between alleles, defined as members of one complementation group, were studied it was found that recombination occurred between many of them, though with a low frequency. The recombinants usually recognised were wild type ones, since in the systems studied these are prototrophs which can be selected with ease through their independence of supplementary nutrients. The actual frequencies of the recombinants depended upon the particular pair of alleles studied and upon other factors that are mainly genetical. The usual order of frequency with which prototrophic recombinants occurred in interallelic crosses of auxotrophs ranges from 10^{-5} or lower to as high as 2.5×10^{-3}. The reciprocal recombinants, combining the differences by which each allele differs from wild type, occur with corresponding frequencies in cases where efforts to observe them were made. However, by reason of technical difficulties, they have rarely been observed.

Recombination between alleles in Neurospora and other fungi, including yeasts, shows a number of special properties. When all products of each meiosis are analysed, it is found that the recombinant is usually not accompanied by the complementary recombinant. Thus the process leading to recombination between alleles is usually not reciprocal in its results, whereas recombination between non alleles is regularly reciprocal.

The general picture of allelic recombination is illustrated by the first exact study made in Neurospora crassa, using crosses between pdx and pdx^P. Each mutant requires pyridoxin for growth, pdx under all cultural conditions, pdx^P only when the medium is on the acid side of neutrality. Each can also be identified by whether or not prototrophic recombinants occur in crosses to standard strains; a double mutant strain would be expected not to yield prototrophs in crosses to either standard.

Although the two pyridoxin mutants do not complement in a heterocaryon and so are alleles (members of the same cistron) by this physiological test, crosses between them yield prototrophic recombinants with a frequency of about 0.2%. The pyridoxin locus is situated between pyrimidine-1 and colonial-4. M. Mitchell (1955) analysed 585 asci, formed by the heterozygote pyr-1 pdx + / + pdx^P co-4, and found that four of them contained one or, in one case, two pairs of spores which were prototrophic recombinants. These asci appear to show a conversion of one or other of the pdx alleles to the normal allele pdx^+. In all cases, there was an absence from the same asci of the pdx pdx^P double mutant. In three asci, the conversions were not accompanied by any change in the combinations of the outside markers; in the fourth conversion and crossing over occurred together.

In striking contrast, crosses between non alleles, even though closely linked (showing rare recombination) have reciprocal recombinants in tetrads and also show correlated recombination between flanking markers. Thus among 646 asci analysed by Giles, deSerres & Barbour (1957) from crosses between ad-3A and ad-3B mutants, six had prototrophic recombinants. Each of the six asci also carried the double mutant combination and there was correlated recombination between the flanking markers his-2 and nic-2, which were also segregating.

Contrasts of this kind at first gave rise to the notion that conversion in allelic recombination is due to a different mechanism from crossing over. Nevertheless the collection of extensive data by tetrad analysis, showed that about half of all tetrads in which there were conversions at the loci studied had correlated recombination between flanking markers, especially where this frequency is low. Representative of such information are the data of Stadler & Towe (1963) for cysteine mutants in Neurospora and, very extensively, of Hurst, Fogel & Mortimer (1972) in yeast. The conclusion is that conversion and crossing over are different manifestations of one basic mechanism, such that half of the events capable of yielding a conversion will contribute a crossing over in the segment within which the event occurs. This conclusion of correlation is reinforced by recent evidence that the frequencies of both events in a given region are affected simultaneously by recombination genes. It is notable that the correlation of crossing over and conversion is also found in Drosophila melanogaster and probably Zea mais. Two other features of conversion are particularly significant. One is the fidelity of conversion indicating that the phenomenon is a highly local one dependent upon the precise interaction of the genetic differences in a particular heterozygote. The second is the occurrence of post meiotic segregation indicating that the process of conversion may not always be completed before the end of meiosis and so involves subunits of chromatids rather than chromatids as wholes.

Mechanical theories of crossing over do not predict conversion nor do they readily account for the precision of recombination in the face of the two stranded structure of DNA. The discovery of genes which control or regulate crossing over and conversion leads, on the contrary, to theories of recombination in which a series of chemical transformations of a pair of chromosomes are mediated by enzymes. Studies of Neurospora crassa have disclosed a number of gene loci with functions in recombination of three general types, distinguished by the action of recessive alleles: (1) a general loss of recombination and often an increased sensitivity to radiations; (2) a local increase of conversion and/or crossing over in

regions commonly not linked to the recombination locus; (3) a local decrease in the immediate vicinity of the gene, provided recessives of the second type are present. The first type is well known in a number of organisms, especially bacteria, while the second type is also known in Schizophyllum. Genetics has the power in these mutants to dissect the mechanism of itself.

Present theories of recombination postulate that it is the consequence of an interaction between two homologous chromatids each consisting of one DNA molecule, namely two complementary nucleotide chains. It involves breakage of chains in each recombining chromatid, the breaks being placed so that associations can occur by annealing of complementary nucleotide chains from the two molecules to form segments of hybrid DNA, hybrid in the sense that the component strands are derived from the homologues. The formation of the hybrid segments may, or may not, be accompanied by recombination of the two DNA molecules to effect a cross over. If the parental chromatids differ by one or more mutations situated in the hybrid segment, a system of enzymes may recognise the resulting distortion in the molecules, excise a segment of the nucleotide chain including the mispaired nucleotide(s) from one strand of a pair and effect repair by synthesising a new segment complementary to the other strand. This is the essence of the theories first enunciated by Holliday (1964) and Whitehouse (1963). The various forms of these theories and their subsequent modifications are difficult to discriminate between experimentally.

It is possible that a full and complete understanding of the mechanism of genetic recombination will be reached first in Neurospora crassa, though yeast could overtake in the race. The present favourable position is due to the wealth of information accumulated about the genetics of recombination in Neurospora and the extensive resources of material now possessed by neurosporologists. The pioneering work of Dodge and of Beadle and Tatum, together with their co-workers, in making this fungus a powerful tool of genetics, is reaching full fructification.

Bibliography

Anderson, E.G. 1925 Genetics 10: 403-17.
Beadle, G. W. & E. L. Tatum 1941 Proc. Nat. Acad. Sci. 27: 499-506.
Benzer, S. 1957 In "The Chemical Basis of Heredity" (Ed. McElroy & Glass) pp 70-93.
Bridges, C. B. 1916 Genetics 1: 1-52 and 107-63.
Dodge, B. O. 1929 Mycologia 21: 222-31.
Giles, N. H., F. J. deSerres, and Evelyn Barbour 1957 Genetics 42: 608-17.
Holliday, R. 1964 Genet. Res. 5: 282-304.
Hurst, D. D., S. Fogel and R. K. Mortimer 1972 Proc. Nat. Acad. Sci. 69: 101-5.
Lindegren, C. C. 1933 Bull. Torrey Bot. Club 60: 133-54.
McClintock, Barbara 1945 Amer. J. Bot. 32: 671-8.
Mitchell, Mary B. 1955 Proc. Nat. Acad. Sci. 41: 215-20.
Pascher, A. 1918 Ber. deuts. bot. Gesells. 36: 163-8.
Stadler, D. R. and Agnes N. Towe 1963 Genetics 48: 1323-44.
Sturtevant, A. H. 1913 J. Exp. Zool. 14: 43-59.
Tatum, E. L. 1946 C. S. H. Symp. Quant. Biol. 11: 278-84.
Whitehouse, H. L. K. 1963 Nature 199: 1034-40.
Wilcox, M. S. 1928 Mycologia 20: 3-16.