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NEUROSPORA AS AN OBJECT FOR CYTOGENETIC RESEARCH

(chromosome rearrangements, crossing over, duplications, meiosis, meiotic drive, recombination)

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SUMMARY

Several lines of research have been stimulated because the direct results of Mendelian segregation are apparent in Neurospora asci. Spore killer genes provide an example. When a cross is heterozygous for Spore killer, four ascospores in each ascus are white and inviable. Surviving ascospores contain the killer allele, which thus shows meiotic drive. Meiotic and postmeiotic divisions are completed before killing occurs.---Chromosome rearrangements are frequent; many have been characterized. Rearrangements are detected initially because they produce white deficiency ascospores. Provisional diagnosis is by visual inspection of asci: the frequencies and patterns of white ascospores are characteristically different for different rearrangement types. When insertional or terminal translocations are crossed by normal sequence, meiotic recombination results in progeny that contain a nontandem duplication. The duplications can provide information on map sequence, dominance, nuclear autonomy, vegetative incompatibility, nucleolus-organizer behavior, mitotic recombination, chromosome stability, and chromosome organization.---Some mutants that impair meiosis produce no ascospores; others produce hypoploid ascospores that are inviable.---Light microscopy has been effective in detailing pachytene chromosome morphology, identifying rearranged chromosomes, and describing the behavior of chromosomes and organelles during meiosis and ascus development in normal and mutant genotypes. Studies of the synaptonemal complex are favored by the small genome size of Neurospora.---Crossing over in Neurospora resembles that in higher organisms, with positive chiasma interference. When gene conversion occurs, flanking markers are recombined with a probability less than 50%, suggesting a constraint on random isomerization in molecular recombination models. Recombination frequencies in specific local regions are precisely controlled by a system of regulatory genes.---Natural populations of Neurospora are readily sampled. Chromosomal polymorphisms have not been detected, al-

though genic polymorphisms are abundant. Karyotypes are similar in all known species.---Beginnings have been made using Neurospora in molecular cytogenetics and for research with recombinant DNA.

INTRODUCTION

What a geneticist does, and how successful he is, depends to no small extent on the advantageous features and limitations of the organism he works with. Examples illustrating this principle were given by STURTEVANT (1971) at the first Stadler Symposium, in his lecture "On the Choice of Material for Genetical Studies."

I propose now to look at Neurospora from this point of view, considering some features of the organism that serve as challenges, and describing some of the responses that geneticists have made. Examples will be taken mostly, but not entirely from experience in my own laboratory, where many of the contributions have been made by my present colleagues EDWARD BARRY, MONIKA BJÖRKMAN, DOROTHY NEWMEYER, NAMBOORI B. RAJU, and BARBARA TURNER, as well as by former associates who are cited in the text.

VISUALIZING THE DIRECT RESULTS OF MENDELIAN SEGREGATION

The pigmented ascospores and linear asci of Neurospora provide a means of visualizing the direct results of Mendelian segregation. If a geneticist were designing an experimental organism ideally suited to reveal the details of meiosis and meiotic recombination, he would specify that the products of individual meioses be kept together, preferably in an ordered array reflecting the successive meiotic divisions. He might ask that the four immediate products of meiosis undergo one additional division, so as to sort out possible differences in information due to recombination events at the half-chromatid level (corresponding to half the double helix at pachytene). All meiotic products should survive and produce progeny. Cytologically, details of chromosome morphology and meiotic behavior should be recognizable by light microscopy, especially during prophase I. All these specifications are fulfilled in Neurospora.

Large numbers of asci, each representing the yield of a single meiosis, can be examined visually in either of two ways. The sexual fruiting bodies may be opened, revealing "rosettes" of asci where each octet of ascospores retains its original order. Alternatively, the fruiting bodies may be kept intact, and large numbers of asci may then be scanned after their component ascospores have been shot out spontaneously onto a collecting surface. The shot asci can be collected as groups of eight ascospores, but each octet has lost its linear order. Some information is lost when the order is randomized, but the loss is trivial for most present-day problems. The unordered, shot asci are easy to isolate, and they are widely used.

Three distinct types of genetic investigation have employed asci as a means of visualizing the direct results of Mendelian segregation. These concern ascospore-color genes, Spore-killer genes, and chromosome rearrangements.

Ascospore-color Genes

Every student is familiar with textbook photographs showing 4:4 segregation of an autonomously expressed ascospore-color mutant. With linear asci, second-division segregation frequencies can readily be determined by inspection, providing a system to investigate the effect of genetic and nongenetic factors on crossing over. This approach was important in Neurospora for showing that crossing over is highly variable in different genotypes, and that the variability is under multigenic control (STADLER 1956; STADLER & TOWE 1962; NAKAMURA 1966; LANDNER 1971).

In other fungi, ascospore color markers have been of major importance for studying gene conversion and other rare recombinational events. (For examples of recent work see PAQUETTE and ROSSIGNOL 1978; KITANI 1978; DECORIS et al. 1978.) In Neurospora, this type of study has not been done effectively for a simple reason. Colorless ascospores rarely germinate, and thus the Neurospora ascospore-color mutants cannot be progeny-tested as is done routinely in Sordaria and Ascobolus.

This apparent shortcoming of the Neurospora ascospore mutants may have been a blessing in disguise, because it has focused our attention on other more novel genetic phenomena. Among the most interesting of these is Spore killer.

Spore killer Genes--an Example of Meiotic Drive

Three chromosomal factors called Spore killer (*Sk*) have been found in different wild Neurospora populations. When a cross is heterozygous for one of the killer alleles, each ascus contains four viable black ascospores and four inviable unpigmented ascospores (Figure 1). The survivors contain the killer allele Sk^K. Spore killer is thus a new example of meiotic drive. That is, Spore killers are genes that "cheat" and perpetuate themselves by other means than environmental selection for fitness (CROW 1979). Spore killer resembles Segregation Distorter in Drosophila, Pollen killer in wheat, and Gamete eliminator in tomato. Neurospora has the practical and aesthetic advantage over these other systems that the effects of meiotic drive can be seen directly and dramatically in every ascus.

Extensive genetic and cytological information has been obtained about Spore killer (TURNER & PERKINS 1979; RAJU 1979). When crosses are homozygous for the same Spore killer allele, all eight ascospores are viable and normal--killing occurs only in heterozygotes. Results are the same when Spore killer is used as male or as female parent, in reciprocal crosses. Killers are specific in their action; that is, Spore killer-2 is immune to killing by Spore killer-2, but Spore killer-2 is not

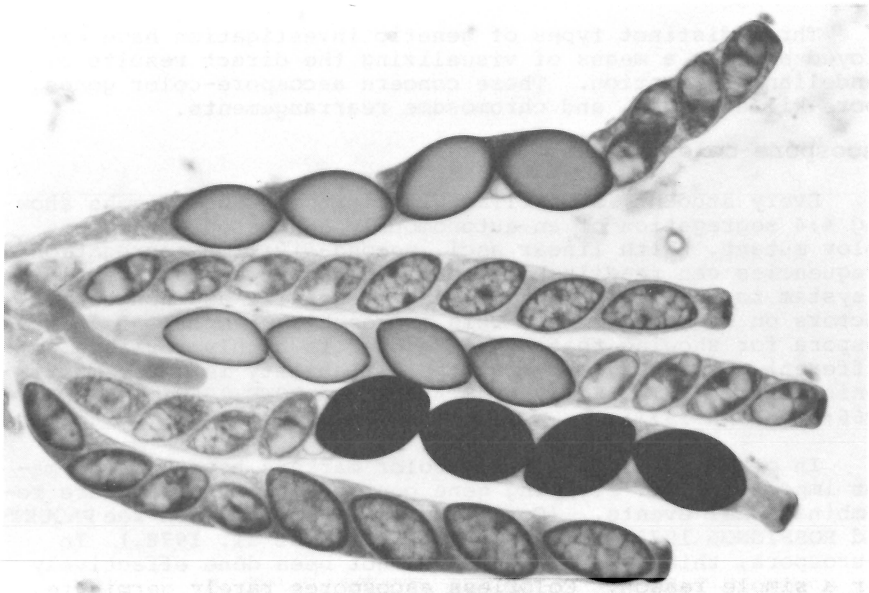

Figure 1. Asci from a cross heterozygous for Spore killer-2. Neurospora asci develop asynchronously, and those shown are at different stages of maturation. The mature (black) ascospores measure about 15 X 29 mμ. (Prepared and photographed by N. B. Raju.)

immune to killing by Spore killer-3, and vice versa. The mapped Spore killer genes are located in the centromere region of linkage group III. The killer alleles are not associated with any gross chromosome rearrangement such as a translocation, and unlinked markers segregate normally when Spore killer is heterozygous. Crossing over is blocked in the vicinity of *Sk*, however, suggesting a local rearrangement. Resistant alleles have been found in natural populations.

In the heterozygous crosses where killing occurs there is no obvious disturbance of meiosis. The two postmeiotic divisions in the ascus are also normal, and killing of a sensitive ascospore normally occurs only after ascospore walls have formed and one mitosis has been completed within the ascospore. Developmental mutants can be used to obtain ascospores that enclose more than one of the meiotic products. If both sensitive and killer nuclei are present in the same ascospore, the spore survives. The sensitive nuclei also survive and can be rescued.

Spore killer presents a challenge on two distinct levels: What are the cellular and molecular mechanisms involved? And what are the role and significance of Spore killer in populations? Neurospora seems to offer advantages for approaching problems at both levels.

Chromosome Rearrangements

Just as was true for Spore killer genes, the study of chromosome rearrangements is enormously aided by being able to observe asci with pigmented ascospores. Both initial detection and preliminary diagnosis of rearrangements can be accomplished by direct visual examination of Neurospora ascospores and asci. Whenever a rearrangement is present in heterozygous condition, pairs of white ascospores appear (Figure 2). The white spores are inviable because they contain deficiencies resulting from segregation and recombination events in meiosis. Different major types of rearrangements can be distinguished because they produce asci having different numbers of black and white ascospore-pairs in characteristic patterns and frequencies (Figure 3).

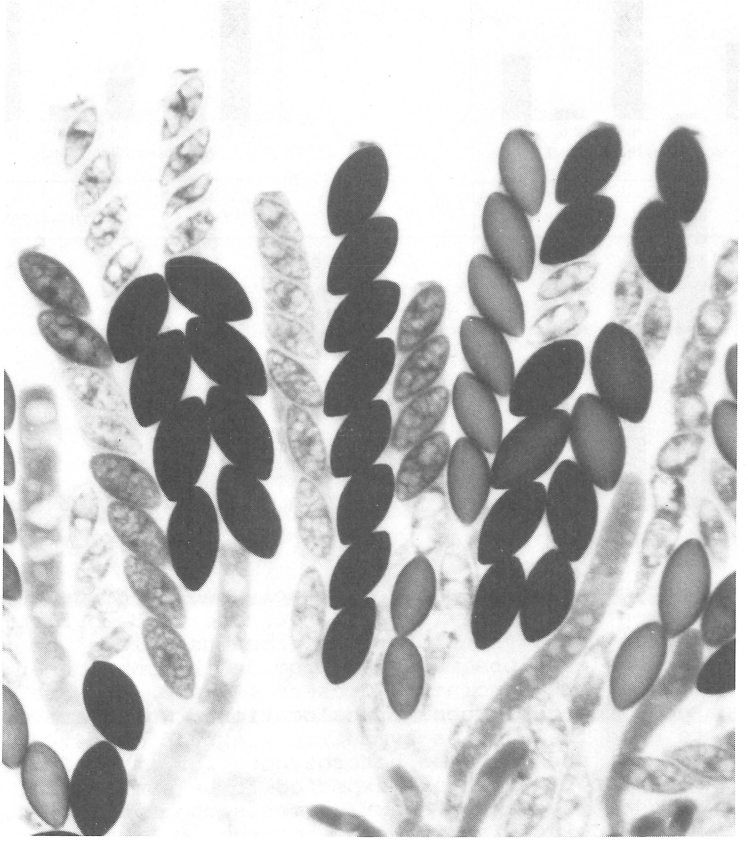

Figure 2. Asci from a cross heterozygous for an insertional translocation. Mature asci are of three types: 8 black : 0 white, 4 black : 4 white, and 6 black : 2 white. (*T(I→VI)NM103* X *Normal*. Prepared and photographed by N. B. Raju.)

Translocations are immediately seen to differ from ascospore-color genes or Spore-killer genes. When a gene is responsible, rather than a translocation, only a single ascus pattern is found, and all asci are uniformly of the type 4 black : 4 white.

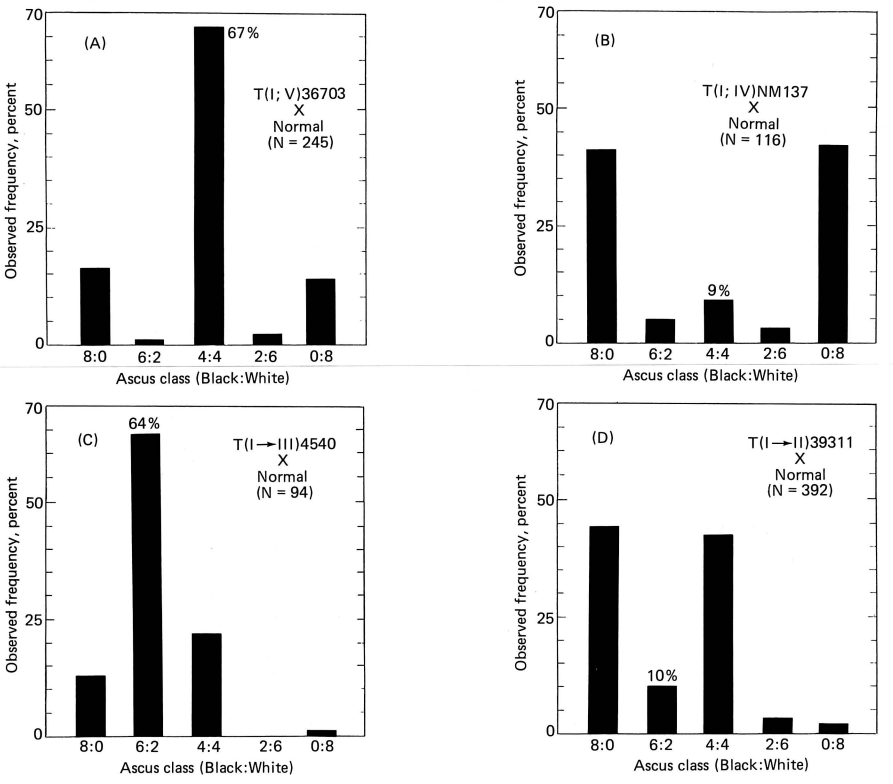

Figure 3. Frequencies of unordered asci used to diagnose rearrangements. Visual inspection can be used to distinguish reciprocal translocations from insertional or terminal translocations, and can provide information on the location of break points. Crosses (A) and (B) were heterozygous for reciprocal translocations, which are expected to give frequencies symmetrical around the 4:4 class. Crosses (C) and (D) were heterozygous for insertional translocations, which are expected to be symmetrical around the 6:2 class. Rearrangements used in (A) and (C) have break points far from the centromere, resulting in high frequencies of the middle class. Rearrangements used in (B) and (D) have both break points close to the centromere, resulting in low frequencies of the middle class. For rationale see PERKINS (1974). (Reproduced from BARRY & PERKINS 1977 with permission of the publisher.)

Neurospora rearrangements and their uses have been reviewed and illustrated elsewhere (PERKINS & BARRY 1977; PERKINS 1974; BARRY 1967; BARRY & PERKINS 1969). I shall indicate here only a few selected results.

In routine mutant hunts following mild UV-treatment, at least 10% of surviving Neurospora cultures contain new chromosome rearrangements detectable by their visual manifestation in the asci. Large numbers of different aberrations have been identified, and nearly 200 have been fully analyzed and mapped. Working with new chromosome rearrangements forces an investigator to become familiar with the entire genome and with all categories of genetic markers. I recommend it as a broadening experience.

A majority of the Neurospora rearrangements are reciprocal translocations, but about one in five is either an insertional translocation or a translocation that involves a chromosome tip. When one of these insertional or terminal translocations is crossed by standard sequence, it characteristically produces a large class of viable progeny that are duplicated for a defined chromosome segment. The duplications are nontandem. Among all the structural alterations identified so far, we have found duplication-producing rearrangements to be the most useful and interesting.

The prominence in Neurospora of rearrangements that produce nontandem duplications suggests an alternate mechanism to tandem duplication, whereby new genetic material might have arisen during evolution.

USES OF DUPLICATION-PRODUCING REARRANGEMENTS

As in other organisms, segmental aneuploids have proved to be extremely useful cytogenetic tools. Duplications are much easier to work with in Neurospora than in most eukaryotes. The main reason is that duplications occur as partial diploids ("partial disomics") against a haploid background, rather than partial triploids against a diploid background, which is usually the situation in higher organisms.

Figure 4 shows segments of linkage group I that can be obtained as nontandem duplications, when any one of 15 rearrangements is used as a parent. For example, when translocation AR190 is crossed by wild type, one third of the viable progeny are duplicated for nearly the entire right arm. When markers are introduced, such a cross performs a "left-right test" by showing whether or not a recessive gene is covered in the duplication progeny, in heterozygous condition (see PERKINS 1972). In other words, precise mapping can readily be done with duplications in Neurospora, comparable to deletion mapping in other organisms.

Our kit of duplication-producers provides a variety of opportunities. In addition to mapping centromeres and genes

by the left-right test, the duplications have been used to study dominance. This is of special interest with regulatory genes (METZENBERG, GLEASON & LITTLEWOOD 1974), where information on nuclear autonomy can also be obtained by comparing heterozygous duplications with heterokaryons of similar gene content. Vegetative incompatibility genes have been identified and studied using duplications (PERKINS 1975; MYLYK 1975). Strains can be produced routinely that contain two nucleolus organizers per nucleus, doubling the normal dosage of ribosomal DNA (PERKINS, RAJU & BARRY 1979). Segmental duplications have provided a means of studying mitotic recombination (BARRY 1978). Altered ability to delete duplications is a frequent property of radiation-sensitive mutants (NEWMEYER, SCHROEDER & GALEAZZI 1978).

Duplication-producing rearrangements are of two main types: insertional and terminal. Both have unique and interesting properties. I'll describe one example that is related to the nature of chromosome ends.

"Terminal" Rearrangements and the Nature of Subtelomere Regions

Translocations involving a chromosome tip are surprisingly frequent (nearly 10% of the aberrations identified in Neurospora fall into this class). We interpret these tip translocations as reciprocal exchanges in which one of the participating chromosomes has contributed only the telomere region. No essential genes can have been translocated with the telomere, because meiotic products lacking the translocated tip nevertheless survive and give rise to viable cultures. These tip-deficient f_1 cultures are simultaneously duplicated for the chromosome segment that was translocated onto the tip.

Terminal duplications from several rearrangements of this type have been studied, and they show unexpected behavior. The duplications are more or less unstable, reverting to the euploid, haploid condition. Reversion occurs by precise deletion of one of the duplicated segments. Loss from either the standard chromosome or the translocation chromosome should be equally effective in restoring haploidy, and there is no *a priori* reason to expect one type of deletion more frequently than the other.

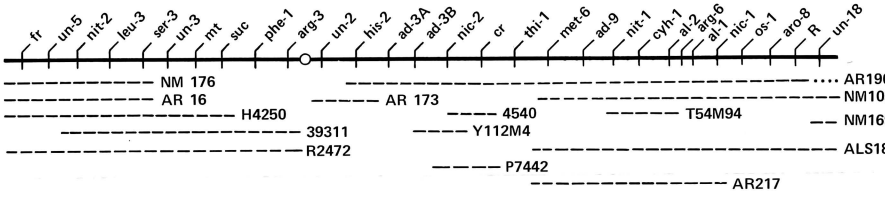

Figure 4. Linkage group I of *Neurospora crassa*, showing the segments (dashed lines) that can be obtained as viable nontandem duplications in progeny of various duplication-producing chromosome rearrangements.

In fact, restoration of haploidy occurs exclusively or predominantly by loss from the translocated sequence, so as to restore normal sequence. This is true of duplications from several different tip rearrangements. (One exception is known.) Deletion is nonrandom, but it is precise.

A model has been suggested that would predict both the nonrandomness and the precision. If regions of repetitive homology exist just proximal to telomeres, and if the original translocation arose by a break within such a region, then some of the repetitive material would still be present at the tip interchange-point (Figure 5). This would allow the duplicated segment to be recombined out of the translocated chromosome, but not out of the standard sequence (NEWMEYER & GALEAZZI 1977). There is cytological evidence in several organisms for terminal or subterminal redundancy of the type required (see, for example, RUBIN 1977).

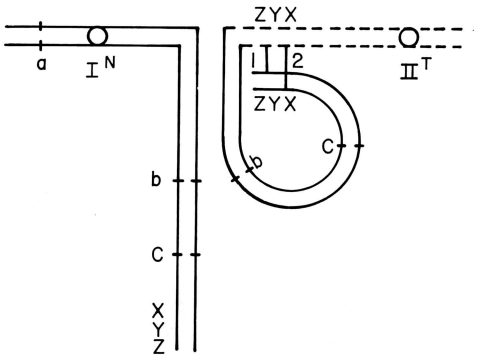

Figure 5. Scheme for deletion of a terminal nontandem duplication preferentially from the translocated position, by recombination involving redundant homologous regions just proximal to the telomere. XYZ represents a tandemly repetitive region, of which only one copy is shown in each position. This mechanism was proposed by NEWMEYER & GALEAZZI (1977).

CHROMOSOME CYTOLOGY AND MEIOSIS

The Pachytene Karyotype

Yet another challenge, compounded of both difficulties and opportunities, has been presented by the small genome of Neurospora, which contains less than 1% of the DNA content of maize (Figure 6). Remarkably, the small DNA content does not preclude being able to see the chromosomes using light microscopy, and to observe many details of chromosome morphology, as first shown by MC CLINTOCK (1945). Neurospora is thus at a crucial position among research organisms. On the one hand, the methods of classical cytogenetics can be applied, as developed in higher eukaryotes. On the other hand, culture methods, selective techniques, and molecular approaches can be used that are typical of haploid microbial genetics. It was this dual challenge that led some of us to persist in genetic and cytological studies of Neurospora during the rise of prokaryote molecular genetics in the 1950's and 1960's.

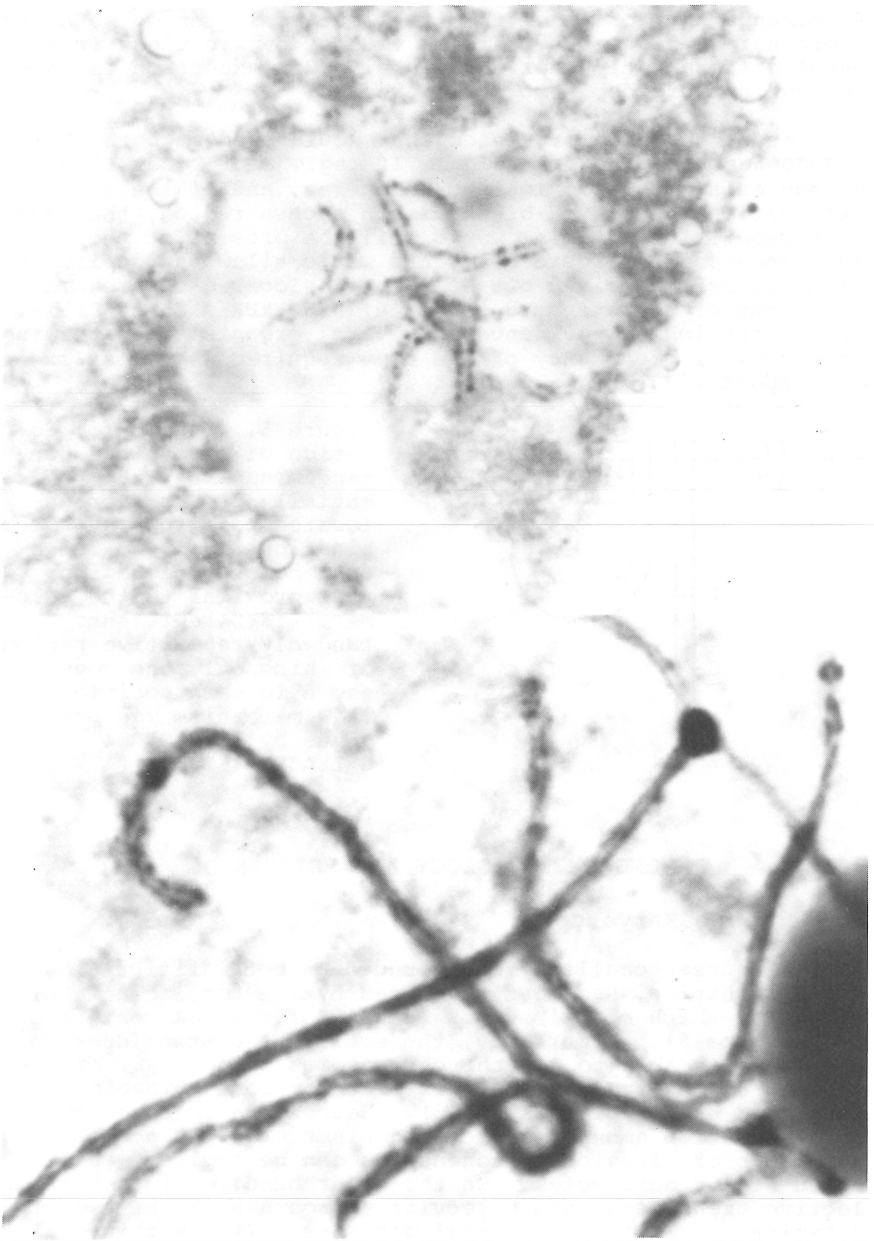

Figure 6. Pachtytene chromosomes of Neurospora above, maize below, shown at the same magnification. (X 3100. Photographed by E. G. BARRY and J. R. SINGLETON.)

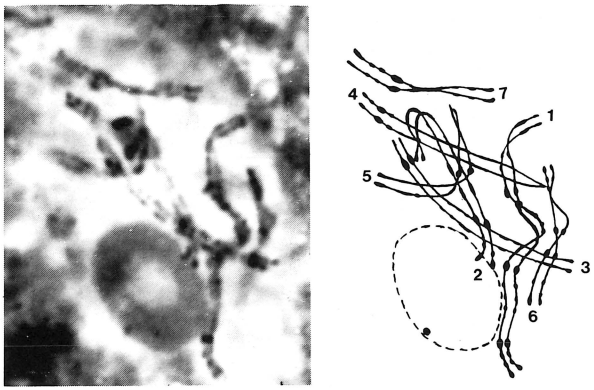

Figure 7. The seven bivalents of *Neurospora crassa* at pachytene. Not all chromosome segments can be seen in a single photograph, although they are readily visible in the microscope, because the focal depth is extremely shallow at the high magnification required. (X 4000. Photographed by E. G. BARRY, and reproduced from PERKINS & BARRY 1977 with permission of the publisher.)

Techniques for studying meiotic chromosomes with the light microscope have gradually been perfected (reviewed by PERKINS & BARRY 1977; see also RAJU & NEWMEYER 1977; LU & GALEAZZI 1978). All seven chromosomes of the pachytene karyotype can be recognized and identified morphologically using conventional light microscopy (Figure 7), even though the basic DNA content of the individual smaller chromosomes is similar to that of E. coli.

When a reciprocal translocation is heterozygous, a classical cross-shaped quadrivalent is formed at pachytene (Figure 8).

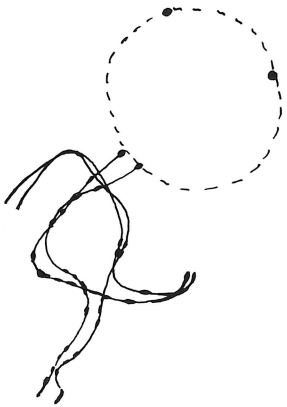

Figure 8. Pachytene pairing of a reciprocal translocation involving the nucleolus-organizer chromosome. The longest chromosome of the complement (chromosome 1 = linkage group I) is interchanged with the nucleolus-organizer chromosome (2 ≡ V). Two satellites are seen as dots on the periphery of the nucleolus opposite the organizers. (*T(I;V)36703* X *Normal*. Drawn by E. G. BARRY from preparation photographed in BARRY & PERKINS 1969.)

It is figures such as this that have enabled each genetic linkage group to be assigned to a specific chromosome.

One of the striking characteristics of pachytene chromosomes is their typical railroad-track appearance. This parallels closely the dimensions and geometry of the synaptonemal complex, which has been studied by GILLIES (1972, 1979) using reconstructions from thin sections. Absolute dimensions of the complex in cross-section are approximately the same in Neurospora as in higher eukaryotes. Dimensions of the complex relative to the amount of chromatin are vastly greater in Neurospora, however. Hence, the Neurospora chromosomes are seen paired as parallel strands.

Working with a small genome has some advantages to compensate for the greater acuity required for observation. For example, reconstruction of the synaptonemal complex karyotype requires far fewer sections in Neurospora and its relatives than in Drosophila or corn. Thus it is feasible to follow in detail the development of the entire complex culminating at pachytene, and its subsequent dissipation (GILLIES 1979; ZICKLER 1977).

Of special interest are the electron-dense recombination nodules that are associated with the central element of the complex. The location of these nodules along the pachytene bivalents of Neurospora and related fungi corresponds to the expected location of reciprocal genetic exchanges (GILLIES 1979; ZICKLER 1977). This confirms Carpenter's discovery of the relationship between spherical recombination nodules and crossing over in Drosophila (CARPENTER 1975; review by MOENS 1978).

In higher eukaryotes it is now possible to obtain entire synaptonemal complex complements with their recombination nodules intact, as water-spread preparations, without sectioning (e.g., MOSES 1977a, b, c; MOENS 1978). Successful application of this technique to the synaptonemal complexes of lower eukaryotes, with their tiny chromosomes, would be a major development. In Neurospora, it would complement present techniques. In other small-genome organisms such as yeast, Chlamydomonas, and Aspergillus, an effective spreading technique for the synaptonemal complex would make pachytene analysis feasible for the first time.

Meiosis and Ascus Development

Our interest is not limited to pachytene chromosomes. Light microscopy has revealed many details of stages from conjugate nuclear divisions in the crozier (where premeiotic S-phase precedes karyogamy--IYENGAR et al. 1977) through the postmeiotic nuclear divisions. Meiosis and ascus development have been well documented photographically (see, for example, RAJU 1978; SINGLETON 1953).

VARIANTS AFFECTING MEIOSIS

Studies of the genetic control of nuclear and cell division and of recombination have been aptly called "the genetics of genetics" (NASH 1973). The regulation of meiosis is of central interest. Some Neurospora mutants affect meiosis by blocking ascus development at specific meiotic or premeiotic stages, so that perithecia contain few or no ascospores (SCHROEDER 1970; RAJU & PERKINS 1978). Other meiotic mutants entail a failure of synapsis, leading to nondisjunction and aneuploidy (SMITH 1975; LU & GALEAZZI 1978). The first type is signalled by absence of ascospores, the second by production of many inviable, white, hypoploid ascospores. Once a mutant is recognized by these gross effects, fine details of its manifestation can be pursued cytologically and genetically.

Genotypes that affect meiosis include not only recessive and dominant point mutants, but also a majority of duplications (apparently without regard to duplication size or genic content) (RAJU & PERKINS 1978). The most interesting genic mutants show a syndrome of overlapping pleiotropic effects, including not only meiotic impairment but also one or more other symptoms such as impaired DNA repair, speeded deletion of duplications, and increased sensitivity to radiation, histidine, and radiomimetic chemicals (SCHROEDER 1970; NEWMEYER & GALEAZZI 1978; NEWMEYER, SCHROEDER & GALEAZZI 1978; KÄFER 1978).

RECOMBINATION

Crossing Over and Interference

Experiments with both tetrads and random meiotic products have shown unequivocally that classical reciprocal crossing over and interference in Neurospora resemble these processes in higher eukaryotes such as Drosophila and maize (PERKINS 1962).

Chiasma interference is positive. Although the cellular and molecular basis of chiasma interference is still not clear, recombination data from Neurospora and other lower eukaryotes offer some promising clues. One crucial observation is that, when gene conversion occurs at a middle locus, flanking markers are usually recombined in *fewer* than 50% of the involved tetrads or chromatids. (The assertion is commonly repeated that flanking markers are recombined with a probability of 50% when conversion occurs. Critical examination of the evidence shows clearly that this is not true [WHITEHOUSE & HASTINGS 1965; STADLER 1973; FOGEL et al. 1978; PERKINS, in preparation]).

The fact that flanking markers are recombined in fewer than 50% of the convertants is not compatible with free isomerization in the molecular recombination model of MESELSON & RADDING (1975). If constraints on isomerization were due to the rigidity and tensile properties of the synaptonemal complex, and breaks of lateral elements of the complex were re-

quired in order for flankers to recombine, as suggested by MOENS (1974, 1978), this might provide a structural basis for both normal chiasma interference and genetically controlled differences in chiasma frequency, localization and interference.

The Precise Regulation of Recombination in Local Regions

A subtle aspect of the "genetics of genetics" concerns genes that control meiotic recombination in specific local regions. These were discovered in Neurospora and examined in a series of elegant studies by D. G. CATCHESIDE and his associates (review in CATCHESIDE 1977). Variants of three classes are known: dominant regulatory genes (*rec*) which act at a distance to reduce recombination in specific target regions, presumably by producing a repressor; an element (*con*) adjoining the target, presumably the site of action of the repressor; and an adjacent *cis*-acting element (*cog*) which is presumably involved in recognizing a general recombinase, and which is dominant in increasing recombination. Information regarding the action of *cog* comes in part from a translocation that has one interchange point inside the target gene, between marker sites employed to assay recombination.

Gene Conversion

The early contributions of Neurospora are common knowledge. Following the original conclusive proof of conversion (MITCHELL 1955), critical information on conversion fidelity and polarity was also obtained in Neurospora (CASE & GILES 1964; MURRAY 1963; STADLER & TOWE 1963). The study of conversion then shifted largely to other fungi where either visual selection was possible as in Ascobolus and Sordaria, or conversion occurred at high frequencies as in yeast (FOGEL, HURST & MORTIMER 1971). A notable exception is the continuing work with Neurospora *rec* genes, which have been examined in detail for their effects on the polarity and frequency of intragenic recombination at various target loci.

OTHER CYTOGENETIC ASPECTS

Natural Populations

Neurospora is widespread in tropical and subtropical habitats. Populations can be sampled by isolating from burned substrates, where each colony originates from a separate heat-activated ascospore (PERKINS, TURNER & BARRY 1976). Over 1000 isolates have been obtained in this way, from hundreds of collection sites, worldwide.

Strains from nature have been a rich source of genetic variants, including both structural and regulatory genes (e.g., BEAUCHAMP, HORN & GROSS 1977; METZENBERG & AHLGREN 1971). *rec* and other genes controlling recombination were also obtained as naturally occurring variants (CATCHESIDE 1975).

Genic polymorphisms in Neurospora populations are similar to those in Drosophila (SPIETH 1975). Polymorphism for chromosome rearrangements has not been detected in Neurospora, however. Translocations have occasionally been found as minority components in individual populations. Inversions may have been overlooked by our methods, however.

All known species of the genus Neurospora are alike in chromosome number (n=7), and similar in chromosome morphology (RAJU 1978; PERKINS, TURNER & BARRY 1976). Interspecific crosses are possible between several of the species, enabling specific genes or chromosome segments to be introgressed from one to the other (e.g., METZENBERG & AHLGREN 1973). Pachytene pairing is close in the one interspecific cross where it has been examined (PERKINS et al. 1976).

Molecular Cytogenetics

After a slow beginning, the stage is now set for more rapid progress in the molecular cytogenetics of Neurospora. Older work was reviewed by PERKINS & BARRY (1977). Histones and chromatin organization in fungi are essentially similar to those of higher eukaryotes (GOFF 1976; MORRIS et al. 1977).

Neurospora DNA fragments have been cloned in E. coli, screened for a variety of purposes, and mapped molecularly (see, for example, GILES et al. 1978; FREE, RICE & METZENBERG 1979; Report on Ninth Neurospora Information Conference, 1978). Use of Neurospora in a host-vector system seems about to be authorized (see Guidelines for Research Regarding Recombinant DNA Molecules, 1978). After long administrative delay, experiments will be permitted that employ recombinant DNA in transformation experiments with Neurospora.

Concluding Remarks

Ten years ago Sturtevant pointed out in his Stadler Symposium lecture what he judged to be two serious difficulties inherent in Neurospora. The usefulness of Neurospora for genetic research was impaired, he said, because "dissection of ascospores from the asci is a very difficult and laborious business, which few people have the skill and patience to carry out...", and because Neurospora is "a difficult and unsatisfactory cytological object." Are these objections justified?

As regards dissection difficulties, not one of the things I have talked about has required that ascospores be dissected out of the asci in linear order. It is regrettable that through the years many geneticists must have avoided using Neurospora because they, like Sturtevant, held the mistaken idea that tedious dissection of asci is an essential part of Neurospora genetics. That is simply not true. Dissection of linear asci is neither required nor common, and in over twenty years of genetic research with Neurospora I have never found it really necessary.

In the beginning, dissection from intact asci was useful for the original mapping of centromeres, and for the first proof of gene conversion (MITCHELL 1955). But for current problems, essential information can almost always be obtained either from random ascospores or from the unordered asci that are shot out spontaneously as discrete octets. And, in special cases such as Spore killer, rosettes of ordered asci can be examined *in situ*, without dissection being required.

Sturtevant's second adverse judgment concerned cytology. I have presented some examples, and cited others, which I hope have convinced you that Neurospora is not unsatisfactory in this respect. True, Neurospora has no polytene chromosomes, and the small DNA content presents a challenge. But, for meiotic-chromosome cytology with the light microscope, Neurospora seems remarkably good, and clearly superior to Drosophila oocytes, even after the advances reported in that organism by PURO & NOKKALA (1977).

I haven't touched on a quite different, but important, facet of genetic research with Neurospora--the human aspect. Characteristics of a research organism determine the pace and style of a laboratory, and to no small extent they also color the life style of the investigator. In this respect I have found Neurospora very congenial. The tempo is comfortable, housekeeping is simple, scheduling is flexible. There are rich genetic resources of mutants, wild-collected strains, and rearrangements. The organism is aesthetic and colorful. There is a pleasant tradition of cooperation among Neurospora workers. There is also a stimulus to keep in touch with prokaryote and higher-organism genetics, and with molecular and classical and population genetics.

In a word, doing Neurospora genetics is not only intellectually rewarding, it is fun.

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