Aspects of Microbiology 12

Microbial Extrachromosomal Genetics

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Van Nostrand Reinhold (UK) Co. Ltd

Published in 1985 by
Van Nostrand Reinhold (UK) Co. Ltd
Molly Millars Lane, Wokingham, Berkshire, England

Photoset in Times 9 on 10pt by Kelly Typesetting Limited
Bradford-on-Avon, Wiltshire

Printed and bound in Hong Kong

ISBN 0 442 30587 7

Preface

The eukaryotic cell is a genetic chimera; it contains structurally and functionally distinct genomes in its nucleus, cytoplasm and cytoplasmic organelles. The phenotype of a eukaryote is dependent on contributions from all of these genomes and on cooperation between them. The elucidation of these separate contributions and their interactions is a fascinating problem in cell and molecular biology, and one in which microorganisms represent important experimental subjects. This book traces the development of our understanding of extrachromosomal genetics in microorganisms from its beginning in the discovery of anomalous, and even bizarre, inheritance patterns to the mechanistic molecular biological analyses of today. It gives special emphasis to mitochondrial and chloroplast inheritance, discusses the evolutionary origin of these organelle genomes and relates this to the development of the eukaryotic state. We hope that this volume will be of use to students of microbiology, genetics, biochemistry, molecular biology and evolutionary biology. In such a brief work we have necessarily made omissions and simplifications, but we hope that some readers will be stimulated to undertake a deeper study of this topic and we have offered some guidance on further reading.

This work has benefited from the criticisms of the series editors and of the referee nominated by the American Society for Microbiology. Most of all it has profited from the continual editorial comment of Rowena Oliver whose insistence that it should be understandable to a zoologist has, we trust, decreased its opacity.

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1 Introduction

Gregor Mendel was either very shrewd or very fortunate in his choice of characters when he studied inheritance in the garden pea. The characters that he chose, such as seed colour and seed shape, proved to be controlled by genes which were carried on different nuclear chromosomes. The behaviour of these genes during mating experiments therefore reflected the behaviour of chromosomes during meiosis (the formation of germ cells) and zygosis (the fusion of the haploid nuclei of germ cells). Thus, Mendel's laws of segregation and independent assortment were a formal description of chromosome behaviour. There are, however, genes which do not obey Mendel's laws because they are not borne on nuclear chromosomes. These are known as extrachromosomal or non-Mendelian genes.

The first description of extrachromosomal inheritance was made by one of the rediscoverers of Mendel's work, the German botanist, Carl Correns. In his pioneering study, Correns employed the flowering plant *Mirabilis jalapa* in his breeding experiments. This is a variegated plant which originated in South America (hence its common name, the Marvel of Peru) and is grown as a perennial in European gardens. The leaves of *Mirabilis* may be of three colour types, green, pale or variegated, and Correns' experiments were designed to study the inheritance of this character. His results, which were published in 1909, are summarized in Table 1. This shows that the inheritance of leaf colour in this plant breaks one of the rules of Mendelian genetics, that the results of reciprocal crosses should be identical. Irrespective of whether a given allele (for instance, that for pale leaves) is donated by the male or the female partner in a cross, its pattern of inheritance should always be the same. Correns' results showed that in *Mirabilis* the contribution of the male partner, the pollen grains, to the inheritance of leaf colour was nil. This trait was solely determined by the female partner. This phenomenon is known as maternal inheritance and is a common one in extrachromosomal genetics. It should be noted, however, that not all cases where different patterns of inheritance are found in reciprocal crosses are due to

Table 1 Inheritance of leaf colour in *Mirabilis jalapa*

Pollen from branch with leaves of type	Pollinate flowers from branch with leaves of type	Progeny have leaves of type
Pale	Pale	Pale
	Green	Green
	Variegated	Pale, green and variegated
Green	Pale	Pale
	Green	Green
	Variegated	Pale, green and variegated
Variegated	Pale	Pale
	Green	Green
	Variegated	Pale, green and variegated

extrachromosomal genes. For instance, sex-linked genes in animals show such non-reciprocity. Therefore the inheritance pattern of a given character must satisfy a number of criteria before we can be confident that it is determined by an extrachromosomal gene. These will be dealt with in more detail later in the chapter.

Since the pollen grain in *Mirabilis* donates little or no cytoplasm to the zygote cell and since it also has no influence on the inheritance of leaf colour it may be concluded that this trait is controlled by a cytoplasmic determinant. The colour of the leaves is determined by the colour of the chloroplasts which they contain. It is now known that the chloroplast is a cytoplasmic organelle which contains a distinct form of DNA (deoxyribonucleic acid) which differs in both its genetical and physical characteristics from the nuclear DNA of the same cell. Moreover, the chloroplast contains its own machinery for the expression, via the processes of transcription and translation, of the genes carried by that DNA.

Chloroplasts are not unique in having their own genetic system distinct from that of the nucleus. This is also true of the mitochondria, a class of cytoplasmic organelle found in all eukaryotic cells. Although chloroplast and mitochondrial DNA encode certain proteins which are essential for the normal function of their respective organelles, they do not contain enough information for the complete fabrication of chloroplasts and mitochondria (Table 2). The construction of these two kinds of organelle is therefore a cooperative effort by the nuclear and the

Table 2 General features of the mitochondria and chloroplasts of eukaryotic microbes, with particular reference to their genetic systems

	Mitochondria	Chloroplasts
Major biochemical functions	Oxidation of carbohydrates, lipids and amino acids, involving the enzymes of the electron transport chain, and resulting in ATP synthesis by oxidative phosphorylation.	Photosynthesis, fatty acid synthesis, nitrite reduction.
Approximate range of genome sizes	20–150 kb	85—possibly 2000 kb
Approximate number of genes carried by the genome.	rRNA: 2 tRNA: 25–35 protein: 10–20	3–5 about 35 50–100+
Proteins known to be encoded by the organellar genome	Mostly components of the electron transport chain: apocytochrome b cytochrome c oxidase (3 subunits) ATPase (2–3 subunits) also 1 ribosomal protein	Many proteins directly involved in photosynthesis: 12–15 thylakoid membrane proteins ribulose biphosphate carboxylase (large subunit) 5 ATPase subunits Also 20–21 ribosomal proteins Protein synthesis factors (e.g. EF-Tu).

organellar genomes. There are also other sites of genetic information within the cytoplasm of eukaryotic cells and the phenotype of such a cell is a composite expression of its nuclear and cytoplasmic genomes. The interaction of these two during growth and division is a fascinating problem in cell biology.

Extrachromosomal genetics has been studied in all kinds of eukaryotes, but microorganisms have become favoured experimental subjects in this branch of genetics as in so many others. They have many features which facilitate genetic studies. Large populations may easily be handled and this permits the induction and selection of rare mutations. Haploid, uninucleate forms occur in some stage of the life cycle of almost all microorganisms which makes detection of nuclear mutations easier. Many eukaryotic microorganisms can be grown and manipulated in the same manner as bacteria, they can be grown in defined media, on agar plates and their colonies may be replicated using velvet pads. All of these features facilitate genetic analysis.

The structural simplicity of many microorganisms commends their use in the study of extrachromosomal genetics. For instance, the unicellular alga, *Chlamydomonas reinhardtii*, has only a single chloroplast and this is one reason for its popularity in the study of chloroplast genetics. The metabolic versatility of microorganisms again commends them for this kind of work. The ability of many green algae to grow heterotrophically on simple carbon substrates as well as autotrophically by photosynthesis is very useful when isolating mutants defective in chloroplast functions. Correspondingly, the budding yeast, *Saccharomyces cerevisiae*, can grow either fermentatively or oxidatively making it the organism of choice for many studies of mitochondrial inheritance. The novel life cycles of a number of microorganisms are also useful. For instance, the heterokaryon stage of many filamentous fungi, in which the cytoplasms of each of the two parents in a cross are mixed but there is no fusion of parental nuclei, may be used to determine whether a given gene is truly cytoplasmic or merely extrachromosomal.

No system is perfect, and perhaps the biggest drawback to the use of microorganisms for these kinds of studies is that many of them have tough cell walls requiring quite harsh methods to break open the cells. This makes the isolation of intact cytoplasmic organelles difficult and cytoplasmic genetics is mainly concerned with organelle genetics.

This brief introduction has given some idea about the nature of extrachromosomal genetics and the reasons why microorganisms are so useful in its study. The rest of the chapter will be devoted to a detailed consideration of the criteria which permit the assignment of a given gene to an extrachromosomal location and to the features of various microorganisms which are of use in making such an assignment.

The first point to make is that we cannot write a list of rigid criteria, all of which must be satisfied in order to assign a particular gene to an extrachromosomal location. Rather, we can supply a list of diagnostic features, more than one of which must generally apply in order to make a confident assignment.

Differences in inheritance patterns between reciprocal crosses

Non-reciprocity has already been discussed with reference to chloroplast inheritance in *Mirabilis*. It was noted then that sex-linked genes in higher organisms also give differing patterns of inheritance in reciprocal crosses and this emphasizes the

fact that this list of features is diagnostic rather than definitive. Correns' experiments with *Mirabilis* were the first published demonstration of maternal inheritance and this is the most common non-reciprocal effect associated with non-Mendelian inheritance.

Maternal inheritance can still occur when the different mating types of a species are not physically distinguishable. Such isogamous matings are quite common among microorganisms and an important example of this phenomenon is chloroplast inheritance in *Chlamydomonas reinhardtii*. In this organism mating is isogamous and there are two mating types, *mt*+ and *mt*−. In crosses, alleles of chloroplast genes carried by the *mt*+ parent are transmitted to all of the progeny of the cross, the *mt*− parent making no contribution. This is an example of maternal inheritance in which the sexes are not morphologically distinct and where there is apparently complete cytoplasmic mixing in the zygote.

Non-Mendelian segregation at meiosis

The rules of Mendelian genetics, as noted earlier, may be regarded as a formal description of chromosome behaviour at meiosis. Many fungi may be used to examine directly the results of meiotic segregation since in these species meiosis produces haploid spores from which vegetative colonies may be derived. The set of four (or multiples of four) spores produced in this manner are referred to as a meiotic tetrad. Two alternate alleles of a chromosomal or Mendelian gene will segregate 2 wild type:2 mutants in such a tetrad assuming that any recombination that occurs is reciprocal. Non-Mendelian genes will not segregate in this manner and therefore a 4 wild type:0 mutants or 0 wild type:4 mutants ratio in the meiotic tetrad is strongly indicative of an extrachromosomal location for the gene being studied.

Somatic or mitotic segregation

The whole purpose of the complex process of mitotic nuclear division is to produce two daughter nuclei which are genetically identical. Therefore there should be no segregation of the alleles of a chromosomal gene during vegetative growth. When different allelic forms do segregate out during mitosis, for instance to produce sectored colonies, then it is likely that the gene concerned is not carried on a nuclear chromosome but is cytoplasmically located. Again, caution should be exercised because sectored colonies can arise due to high frequencies of reversion to wild type or from non-reciprocal recombination events in diploid nuclei.

Gene transfer in the absence of nuclear fusion

This is a particularly useful test since it can show whether a given determinant is not only extrachromosomal but is also extranuclear. The demonstration of gene transfer without the fusion of nuclei is most readily achieved in the fungi. This can be done by following the 'invasive' or 'infective' spread of a character during mating when cell fusion but not nuclear fusion has occurred. In such an experiment a character carried by only one parent can be retrieved by culturing parts of

the mycelium of the other parent which were not directly involved in hyphal fusion and nuclear exchange. An example is the infective spreading of senescence between mycelia of the ascomycete, *Podospora anserina*.

The second method of demonstrating gene transfer in the absence of nuclear fusion that may be exploited in fungi is the establishment of a heterokaryon. In many members of the higher fungi, the *Ascomycetes* and *Basidiomycetes*, the mating of two compatible haploid mycelia leads to plasmogamy, total cytoplasmic mixing and nuclear migration. Thus, in the new mycelium formed, which is called a heterokaryon, the two parental types of nuclei coexist in a common cytoplasm but never, or very rarely, fuse and thus cannot exchange genes (Figure 1). In the *Ascomycetes* the proportions of the two kinds of nuclei in different parts of the heterokaryotic mycelium may vary. However, in the *Basidiomycetes* a dikaryon is formed where the hyphae are divided by special septa into segments which each contain one of each parental type of nucleus. Heterokaryons produce vegetative spores which contain a single nucleus of either parental type surrounded by the common cytoplasm. Therefore, any character donated by only one of the parents to the heterokaryon, which appears in all the progeny grown from its spores must have been transmitted through the cytoplasm.

Absence of linkage to known nuclear genes

The nuclear chromosomes of most eukaryotes can be visualized cytologically and also defined genetically as a number of linkage groups. However, the chromosomes of a number of microfungi such as *Saccharomyces* (yeast) and *Ustilago* (smut) have never convincingly been demonstrated microscopically but it is always possible to assign any nuclear gene to the linkage group which defines, in genetic terms, the chromosome on which it lies. It follows that an extrachromosomal gene should not show any linkage to known nuclear genes. Such lack of linkage is readily demonstrated in species which have a well-defined nuclear genetic map in which markers in the centromere-linked genes of all the chromosomes are available. This emphasizes the fact that it is much easier to do extrachromosomal genetics in species in which many of the chromosomal genes have been characterized.

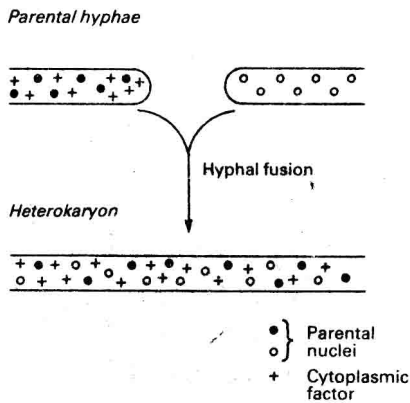

Fig. 1 Heterokaryon formation.

Specific mutational effects

A suggestive, but by no means conclusive, indication that a given mutation is a change in a non-Mendelian genome is given if that mutation can be induced by an agent which is not known to affect nuclear genes. One example is the induction of mutations in the chloroplast genome of *Chlamydomonas* by streptomycin, an inhibitor of protein synthesis on 70S ribosomes, or fluorodeoxyuridine. Others are the induction of the cytoplasmic petite mutation in yeast by the DNA intercalator ethidium bromide or the uracil analogue 5-fluorouracil and the induction of more limited mutations in the yeast mitochondrial genome by Mn^{2+} ions. The specificity of such an effect is confirmed if the agent induces mutations leading to a loss of function in strains which are nuclear diploids or tetraploids. A null mutation in a nuclear gene would be recessive and so not phenotypically expressed in such strains.

Correlation of a given character with the presence of some physical entity distinct from the nuclear chromosomes

In most cases an extrachromosomal genome is first defined genetically and then a search is made for its physical basis. The killer phenomenon in *Paramecium* was recognized as a heritable characteristic and later correlated with the presence of the kappa particles in the cytoplasm. Similarly, the cytoplasmic petite mutation in yeast was well known before being correlated with the loss or severe alteration of mitochondrial DNA. Occasionally biochemists discover a self-replicative molecule in the cytoplasm before geneticists have recognized any phenotype with which it is associated.

This is the case with the 2 μm circular DNA of yeast. On other occasions it is the biochemists who fail the geneticists. For instance, the *psi* factor of yeast, a non-Mendelian determinant which modifies the activity of suppressor genes, has yet to be assigned to any self-replicative molecule.

Experimental organisms

Now that the various tests which can be applied to extrachromosomal genes have been discussed it is necessary to consider which microorganisms are most suitable for the application of these tests.

Algae The study of chloroplast inheritance has been largely confined to unicellular algae, such as *Chlamydomonas* and *Euglena*, and to variegated flowering plants, such as *Pelargonium*. The unicellular algae offer all the usual advantages of microorganisms and a significant advantage of *Chlamydomonas* and *Euglena* is that they are facultative phototrophs, being able to grow in the dark on simple carbon sources such as acetate as well as photosynthetically in the light. To obtain null mutations affecting a given physiological system (in this case photosynthesis) it is helpful if the organism is able to grow and divide even when the system is inactive.

The unicellular, biflagellate alga *Chlamydomonas reinhardtii* has been the principal microorganism used for the study of chloroplast genetics and its life cycle

will be considered in some detail. When grown in liquid culture *Chlamydomonas* is a motile cell bearing two, equal-sized flagella. However, when grown on an agar plate the cells lose their flagella and divide to form discrete colonies. The cell has a rigid cellulose wall which must be ruptured before subcellular organelles or high molecular weight nucleic acids can be isolated. It has a large single chloroplast which cups the nucleus of the cell. Genetic analysis has defined 17 linkage groups within this nucleus, but microscopic visualization of chromosomes is difficult, as it is with many microbial eukaryotes. The nuclear genetics of *Chlamydomonas* is quite well developed. It was the first organism to be used in tetrad analysis (by Pascher in 1916), but the study of chloroplast genetics, initiated by Sager in the early 1950s, has been the real impetus behind the development of its nuclear genetic system. More recently, *Chlamydomonas* has been used to study the genetic control of the cell cycle and this has led to an even more detailed analysis of its nuclear genome.

The life cycle of *Chlamydomonas reinhardtii* is shown in Figure 2. If the motile, vegetative cells are deprived of nitrogen for 2–4 hours, they differentiate into gametes and when gametes of opposite mating types (mt^+ and mt^-) are mixed, they immediately clump together. Pairs of cells of opposite mating type then fuse to produce binucleate, tetraflagellate zygotes. When this mixture is then plated onto agar no further matings are initiated but the zygotes which have already formed lose their flagella and lay down a thick zygospore wall, at the same time doubling their size. Nuclear fusion follows 3.5 hours later after which meiosis occurs, taking about 12 hours. Spore maturation is completed in approximately 6 days and the mature spores will germinate if transferred to fresh agar medium and placed in the light. The zygote cell wall ruptures to release either 4 or 8 haploid cells depending on the strain used. The meiotic tetrad may be analysed either by a 'self-dissection' process which uses water drops to disperse the four spores or by more conventional micromanipulation.

Nuclear genes, such as the mating type locus, segregate 2:2 in this meiotic tetrad. Chloroplast genes are inherited in a uniparental fashion reminiscent of

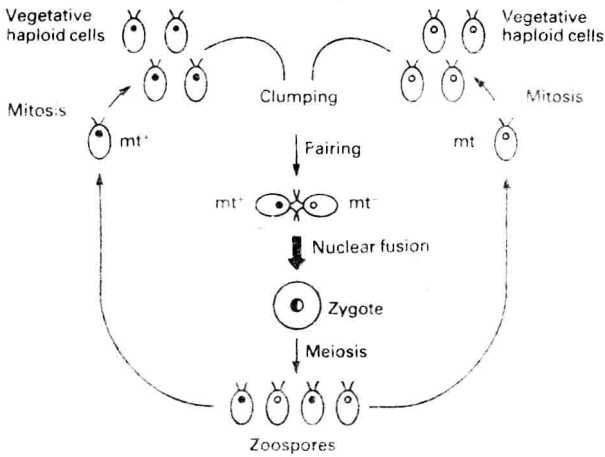

Fig. 2 Life cycle of *Chlamydomonas reinhardtii*.

maternal inheritance in higher plants. In over 90% of tetrads the chloroplast genotype of the mt^+ parent, hence referred to as the maternal partner, is inherited by all the four spores in the tetrad. This feature greatly facilitates the identification of the chloroplast genes. Unfortunately, it also prevents their further genetic analysis because no recombinants may be formed between the maternal (mt^+) and paternal (mt^-) genomes. Some means were needed for increasing the occurrence of the rare phenomenon of biparental transmission in which the zygotes produced from a cross can give rise, by meiosis, to haploid cells containing both the maternal and paternal chloroplast genomes. The two different genomes may recombine and segregate during the succeeding mitotic divisions. Cells containing both parental chloroplast genomes are hence cytoplasmic heterokaryons, and are also known as heteroplasmons or cytohets. Sager and Raminis found that the proportion of biparental zygotes could be increased from much less than 10% to about 50% by the irradiation of the mt^+ parents with ultraviolet light immediately before mating. Heteroplasmons are therefore produced at sufficient frequency to permit the study of chloroplast recombination and the construction of genetic maps for the plastid genome. Sager also found that mutations could be selectively introduced into the chloroplast genome by using high concentrations of either fluorodeoxyuridine or streptomycin so that a detailed genetic map could be constructed.

Table 3 shows that six of the seven diagnostic tests for the identification and analysis of extrachromosomal genes may be applied to the chloroplast system of *Chlamydomonas* which make it a near-perfect system for studying extrachromosomal genetics; considerable progress has been made with this organism.

Protozoa The protozoa are difficult organisms with which to work, mainly because bacteriological techniques are not easily applied to them. Nevertheless,

Table 3

Diagnostic test	*Chlamydomonas reinhardtii*	*Paramecium aurelia*	*Saccharomyces cerevisiae*	*Neurospora crassa*
Differences between reciprocal crosses	+	−	−	+
Non-Mendelian meiotic segregation	+	−	+	+
Mitotic segregation	+	+	+	+
Gene transfer in the absence of nuclear fusion	−	(By micro-injection)	±	+
Absence of linkage to nuclear genes	+	−	+	+
Specific mutational effects	+	(By *in vitro* mutagenesis of killer particles)	+	+
Correlation with some entity other than nuclear chromosomes	+	+	+	+

because they are 'true' animals, they may be claimed with some justification to represent a better model for the human cell than most microorganisms. Moreover, since most protozoa do not possess a rigid cell wall, subcellular organelles may be isolated from them with relative ease.

The ciliate, *Paramecium aurelia*, has been very important in the study of extrachromosomal genetics. This organism, like many protozoans, contains both germ-line and somatic nuclei. It has a single macronucleus, containing several hundred times the normal haploid complement of DNA and this controls general growth and development. Each *Paramecium* also contains two diploid micronuclei. These are the germ-line nuclei, involved in sexual reproduction, and giving rise to the somatic macronucleus.

Sexual reproduction in *Paramecium aurelia* occurs by conjugation between individuals of compatible mating type: the process is outlined in Figure 3a. Sexually compatible strains of *Paramecium* define a number of subspecies known as syngens. *Paramecia* may be induced to conjugate by nutrient deprivation. Contact between a pair of compatible cells (gamonts) initiates the breakdown of their respective macronuclei while each of the two micronuclei in each cell undergo meiosis to produce a total of eight haploid nuclei per gamont. Seven of these nuclei then disintegrate. The remaining nucleus in each cell undergoes a single mitotic division and one daughter nucleus from each gamont migrates into

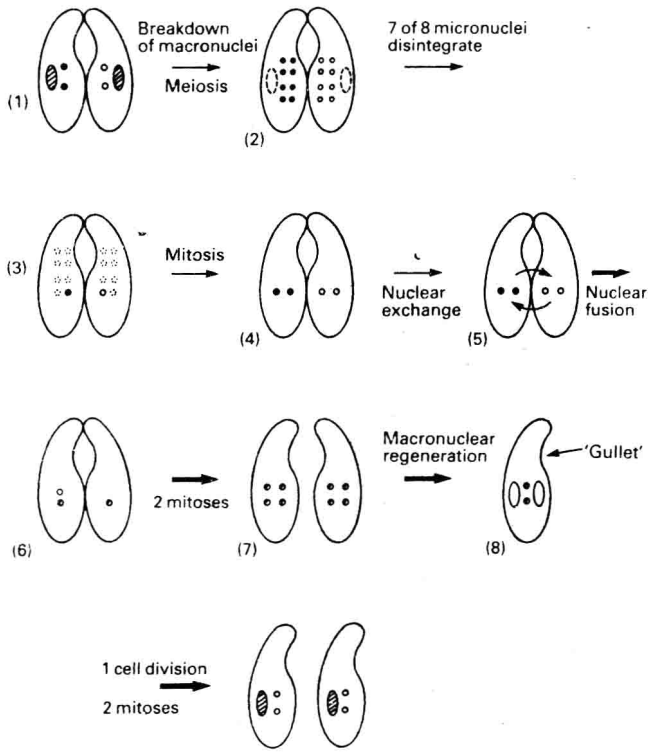

Fig. 3a Conjugation in *Paramecium aurelia*.

the cytoplasm of the other partner through cytoplasmic bridges which have been established in the gullet region (Figure 3a). Each of the migratory nuclei then fuses with the stationary nucleus in its partner gamont to re-establish the diploid number of chromosomes. In addition to this exchange of nuclei during conjugation there may, in some strains, be a fairly extensive exchange of cytoplasm between the mating partners if they are not immediately separated after nuclear fusion.

Separation of the mating partners is followed by two rounds of mitotic nuclear division giving four diploid nuclei, two of which develop into macronuclei and two remain as micronuclei. The two micronuclei each undergo a further mitotic division which is followed by a cell division. The two daughter *Paramecia* now have the usual complement of two diploid micronuclei and one macronucleus.

Paramecium may by-pass the sexual process of conjugation by a parasexual mechanism known as autogamy (see Figure 3b). Autogamy is essentially the same as conjugation except that two haploid nuclei are generated and fuse within a single cell, no sexual partner being involved. For the geneticist, it is a useful method of rapidly establishing homozygous cell lines.

Reference to Table 2 shows that *Paramecium* is not such an amenable subject

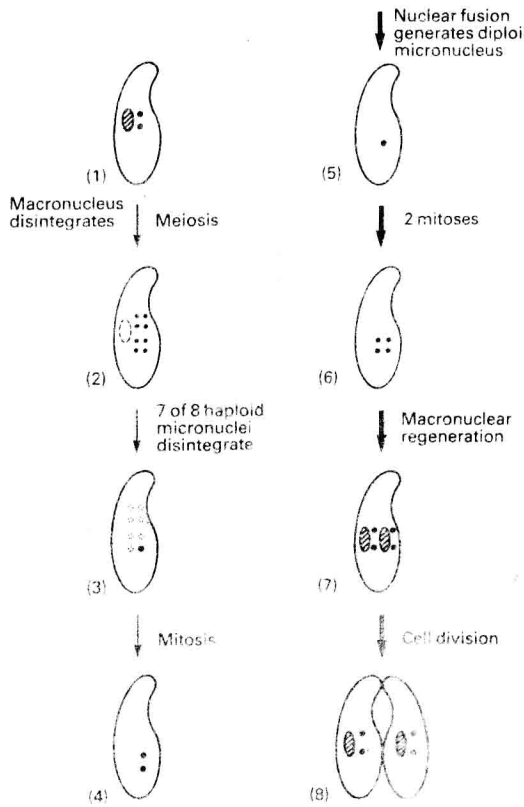

Fig. 3b Autogamy in *Paramecium aurelia*

for research into extrachromosomal genetics as *Chlamydomonas*. Only three of the seven diagnostic tests may easily be applied to it. In order to permit nuclear exchange in the absence of cytoplasmic mixing it is necessary to separate the conjugating pair early in the mating process. The large size of the *Paramecium* cell permits the introduction of either mitochondria or killer particles by the process of microinjection. This allows these genomes to be exposed to mutagens *in vitro* quite independently of the nuclei. These techniques are very powerful ones and confirm the continued usefulness of this organism in spite of its disadvantages.

Fungi The fungi have proved to be the most useful microbial eukaryotes for genetic studies. They may be grown on defined media on simple carbon substrates and are also sexually very versatile, permitting a wide range of genetic techniques to be employed. In terms of the experimental techniques which may be applied to them the fungi may be divided into the yeasts and the filamentous fungi. The yeast *Saccharomyces cerevisiae* and the filamentous ascomycete *Neurospora crassa* (the pink bread mould) have both been extensively used in studies on extrachromosomal inheritance and their life cycles will be examined as representatives of these two groups of fungi.

Saccharomyces cerevisiae, the bread and ale yeast, is a unicellular ascomycete which divides by budding. The unicellular habit of yeast means that it may be handled exactly like bacteria and this commends it as an experimental subject. However, the principal advantage of *S. cerevisiae* in the context of mitochondrial genetics is that, unlike other fungi, it is not an obligate aerobe but may grow and divide using an entirely fermentative metabolism, dispensing with the functions of oxidative metabolism supplied by the mitochondria. This means that mutations in mitochondrial genes are not lethal. Indeed, the organism can still grow and divide on a fermentable substrate even when the entire mitochondrial genome has been lost.

The life cycle of *S. cerevisiae* is described in Figure 4. The organism can grow and divide in either the haploid or the diploid form permitting the identification of nuclear mutants in haploids and the definition of functional genes by complementation testing in the diploids. If the diploid form is subjected to nitrogen starvation it will undergo meiosis to produce four haploid ascospores. These four spores may be separated by microdissection and this permits tetrad analysis to be carried out. Alternate alleles of a given nuclear gene, for instance the a and α forms of the mating-type gene, segregate 2:2 in the meiotic tetrad, i.e. $2a{:}2\alpha$, whilst extrachromosomal genes segregate 4:0.

The diploid state is reconstituted by the fusion of an a cell with an α cell. Nuclear fusion (karyogamy) generally follows immediately after cytoplasmic fusion (plasmogamy) and thus there is no stable heterokaryon formed to allow the distinction between nuclear and cytoplasmic genes. However, in some strains, nuclear fusion is delayed and this permits the isolation by micromanipulation of haploid buds containing a mixed cytoplasm. This heterokaryon test is the only one of the seven diagnostic tests which presents any problems in *Saccharomyces*. Yeast is therefore a near perfect system for the study of mitochondrial genetics. Its utility may be compared to that of other organisms by reference to Table 3.

Neurospora crassa, like *S. cerevisiae*, is an ascomycete fungus which produces haploid ascospores (eight in this case) within a sac called an ascus. The ascus of *Neurospora* is elongated and the linear arrangement of the eight spores within it reflects the relationship between sister chromatids at meiosis I. Such an ordered