Protozoa and genetics

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This paper is a discussion of the value of protozoa as experimental organisms for the study of certain genetic problems. A number of examples of ‘cytoplasmic heredity’ are considered, some being based on DNA-containing particles, and others lacking such a material basis. Examples of the first type are the endosymbionts, such as kappa, and mitochondria; an example of the second type is the system of antigenic variation in Paramecium.

Brief mention is made of some studies on nucleo-cytoplasmic interactions in Amoeba, of the kinetoplast in Trypanosoma, and of some studies on the genetics of malaria parasites (Plasmodium berghei and related species).

As a final example of the value of protozoa in experimental research, reference is made to some behavioural mutants of Paramecium and the bearing of this work on some neurophysiological problems is indicated.

Introduction

In this lecture I would like to discuss the value of protozoa in genetical research. It is obvious that protozoology and genetics are two very different subjects. Protozoology is concerned with an extraordinarily varied assortment of organisms, the only common feature of which is perhaps that they are all unicellular animals. Even that statement may not be acceptable to everyone, since the ‘cells’ of some protozoa have been claimed by botanists as plants. At least all protozoa are eukaryotes and share many features of other eukaryotic organisms, though in other respects they show tremendous variation. Some have characteristics setting them apart from practically all other groups of organisms. For example ciliates contain – in addition to the ‘conventional’ diploid micronuclei – a macronucleus, which is a large structure whose detailed organization is still not clearly understood though it is known to contain many sets of the haploid genome. Ciliates also have an extraordinarily elaborate surface, comprising cilia of course, but also many other structures arranged in exceedingly precise and elaborate patterns. Some flagellates have a kinetoplast – a conspicuous DNA-containing body unlike anything known in higher organisms. Some protozoa, such as malaria parasites, pass through a whole series of distinct stages; others like Paramecium look much the same (at least from the outside) at all times, apart from variations in size due to variation in the
availability of food. Structurally, nutritionally, cytologically and in the ecological
niches they occupy—protozoa show a tremendous diversity and protozoologists
have to deal with this mass of material. Even Leeuwenhoek with his primitive
lenses described a considerable number of different forms. Protozoology is perhaps
still rather an old-fashioned subject but that adds to its charm.

Genetics is very different. It is thoroughly up-to-date, especially in its molecular
aspects, and has a precise theoretical basis applicable to all forms of life, from
viruses to mammals. Some developments, such as genetic engineering, are so
modern as to provide suitable material for science fiction.

In this lecture I plan to select a few examples of work on protozoa to illustrate
their value to genetics, and secondly to show how protozoology may be advanced,
in regard to both theoretical and applied aspects, by genetic work. From the
outset let us admit that work with protozoa has not been productive of results
establishing the basic theory of genetics, as currently understood. Mendelism, the
chromosome theory, the structure of DNA and the many subtle complexities of
molecular biology—all these have developed without any assistance from protozoologists. On the contrary it may have seemed that protozoologists have, if
anything, been a hindrance by bringing up from time to time awkward matters
which did not seem to fit in with the standard beliefs of geneticists. I will mention
a few examples. However, though sometimes irritating, the airing of these unorthodox findings may have had a beneficial effect: if incorrect they can be
rapidly disposed of, but in some cases they result in new ways of looking at old
problems, and suggest new types of research.

The first ‘discrepancy’ comes from Jollos (1921) who proposed the notion of
‘Dauermodifikationen’, based mainly on his work with Paramecium. Jollos found
that organisms could be changed by exposure to particular environments, such as
presence of arsenic or other poisonous substances in the medium, or to high
temperature. As a result variants arose which were better able to resist the harmful
effect of these particular components of the environment. Some changes of this
kind were found to persist for long periods. In a sense they were hereditary but
they certainly did not comply with Mendel’s principles. They even seemed to give
support to Lamarckian views. In later work Jollos had some difficulty in repeating
his experiments, but similar adaptations to certain environmental conditions can
readily be obtained in protozoa and other unicellular organisms (Beale 1953). The
material basis for these adaptations however is not understood.

As a second example of genetically unorthodox findings coming from the work of
protozoologists, we may consider Lwoff’s (1949) notion of the ‘continuité génétique’ of kinetosomes (ciliary basal bodies). These organelles, which are just about
at the limits of resolution with the light microscope, were thought to ‘divide’ or
at least to induce the development of replicas in the immediate vicinity of preexisting kinetosomes. They seemed to constitute an additional, non-nuclear,
genetic system in the cell surface. There have been reports that they contained
DNA, but more recent studies show that the evidence for that was unconvincing.
Kinetosomes can now be shown by electron microscopy to be complex differentiated structures (see, for example, the description of the basal bodies of *Paramecium* by Dippel 1968), and therefore could not divide in the way a strand of DNA replicates. Nevertheless Lwoff’s observations, bearing in mind the limited resolution of the light microscope, were accurate, and laid the basis for much later work on the determination of surface patterns in ciliate cells (Beisson & Sonneborn 1965; Nanney 1968).

A third controversial issue was raised by Sonneborn (1938, 1943) when he discovered the ‘killer’ strains of *Paramecium*. These showed a novel type of inheritance due to a cytoplasmic factor denoted ‘kappa’. Here again there seemed to be evidence for a non-Mendelian genetic determinant. This discovery caused a lively discussion about the existence not only in *Paramecium*, but in all other organisms, of cytoplasmic factors which were called by some workers ‘plasmagenes’. More conventional geneticists opposed the idea of a novel cytoplasmic system of heredity, held firmly to the doctrine of the monopoly of the nucleus and interpreted kappa not as a plasmagene but as a symbiotic microorganism (see, for example, Altenburg 1948). Kappa and many other similar particles are now known to be quite common in ciliates (as well as in other groups of organisms), and are now considered to be bacterium-like endosymbionts. Kappa itself has even been dignified with a Linnean binomial label – *Caedobacter taeniospiralis* (Preer *et al.* 1974). Such particles are nevertheless of interest to geneticists in a number of respects, as will be discussed later.

We now see that none of the three above-mentioned apparently unorthodox genetic phenomena pose a serious threat to current genetic principles, according to which the main genetic apparatus comprises DNA structures in chromosomes in nuclei. These observations do however show that there are additions to this basic apparatus. Outside the nucleus there are auxiliary genetic systems whose relative importance is a matter for discussion. Their study has led to research revealing and clarifying previously obscure areas of biology. One such area concerns the nature and hierarchical relationship of various types of biological unit, such as genes, viruses, organelles, cells and organisms. Another has to do with the capacity of cells and subcellular structures to direct the development of daughter cells or structures. It seems to me that the most valuable contributions of protozoa to genetics (in a broad sense), have lain in these areas. Moreover studies with protozoa illustrate very well what might be described as the ‘untidiness’ of the biological world, as contrasted with the physical world, a statement which I hope will be justified by what is described in this lecture.

The material to be discussed here will be arranged in the following way. First some examples of cytoplasmic heredity in protozoa will be considered, classified in two groups: (i) those based on DNA-containing cytoplasmic particles, such as endosymbionts and mitochondria, and (ii) those not based on any visible cytoplasmic particles, but due to some less clearly specified cytoplasmic processes, called by some, like Nanney (1958) ‘epigenetic’, though since that word has been
used by others with different meanings, it may be safer to use a vaguer term such as ‘developmental’. Examples of the second group include (a) the surface antigens of *Paramecium*, to be discussed later (p. 21), (b) patterns of surface basal bodies and other structures in many ciliates (Beisson & Sonneborn 1968; Nanney 1968; Sonneborn 1974a), and (e) the ciliate mating types (discussed for *Paramecium* by Sonneborn 1974b). In addition a number of important phenomena long studied by protozoologists, such as senescence and rejuvenescence (see Jennings 1929) may also have a quasi-genetic basis (see Sonneborn 1960) though this will not be discussed here.

I will then consider a miscellaneous assortment of studies on different protozoa, including *Amoeba* and malaria parasites, finally returning to *Paramecium* to discuss some recent work on the genetics of behavioural characteristics.

*Paramecium* is the organism more used than any other in genetic work on protozoa. This is mainly the consequence of the discovery by Sonneborn (1938) of the system of mating types in *P. aurelia*, now regarded not as a single species but as a group of distinct eco-species (Sonneborn 1975). Controlled matings and precise genetic analysis, including study of the role of cytoplasmic genetic factors, became possible in *P. aurelia*, which has in the intervening years been much exploited by Sonneborn and members of his school.

In a single lecture it is impossible to refer to more than a small fraction of the material on protozoan genetics available, whether in *Paramecium* or in any other protozoon. The choice of topics here is of course a personal one. A major omission, for which I apologize, is the extensive work on another ciliate – *Tetrahymena pyriformis*.

**Endosymbionts**

By endosymbiont is meant here an organism living inside the cells of another, and use of the term is not meant to imply any necessary benefit to either member of the partnership. However, so intimate an association is hardly likely to exist without causing some mutual interaction. The kappa particles in *P. aurelia*, which are responsible for producing the ‘killer’ paramecia (Sonneborn 1938), and many other similar particles, are now considered, as already stated, to be bacteria. Preer et al. (1974) have listed a number of bacterial characteristics of kappa particles, such as, for example, their size and shape, Gram-negative staining reaction, absence of a nuclear membrane, presence of both DNA and RNA, bacterial-type of ribosomal RNA, capacity to show glycolysis or the pentose-phosphate shunt, etc., etc. Perhaps the only ‘non-bacterial’ characteristic of kappa is its restriction to the cytoplasm of *Paramecium* as a site where growth of the particles can go on. Hence kappa does not fulfil Koch’s postulates, to which all good microbiologists still pay due respect. However, some other endosymbionts of *Paramecium* (those denoted lambda and mu) have been reported to be capable of growing slowly in an external medium outside their normal host and even of infecting a new *Paramecium* (van Wagendonk et al. 1963; Williams 1971).
One remarkable constituent of kappa particles (see Preer et al. 1974) – or at least of those denoted ‘brights’ – is a virus-like element (or ‘plasmid’), containing its own specific DNA. These virus-like particles may be considered ‘defective’ in that, unlike true viruses, after infecting and possibly killing a Paramecium, they do not give rise to a new crop of virus particles. Other endosymbionts in Paramecium (mu, lambda, etc.), some of which act as ‘killers’, do not show any visible indications of viruses, however.

In the early accounts of kappa and other symbionts, much significance was placed on the necessity for the host Paramecium to contain certain supporting genes (K for kappa, M for mu, etc.). Later a detailed theory (the ‘metagon’) was devised whereby these genes supported the corresponding symbionts (Gibson & Beale 1962). This has now been abandoned, due to failure to reproduce the detailed results of experiments on which the theory was based; and the rôle of the supporting host genes in regard to maintenance of the symbionts is still not understood.

Nothing is known about the genetics of the symbionts themselves, since recombinational mechanisms like those which make possible genetic studies with E. coli and other bacteria have not been studied, and may not exist with these symbionts. However, a number of variants of some symbionts are known. For example, different kappa variants are distinguished by their characteristic prelethal effects on sensitive paramecia, and these variants can all be maintained in paramecia of the same genotype, and under similar environmental conditions. It seems reasonable to conclude that most if not all of the properties of the symbionts are controlled by the symbionts’ own DNA, and perhaps by that of the ‘virus’ DNA, when such ‘viruses’ are present.

The endosymbionts of Paramecium may be considered as representatives of a kind of supernumerary, optional, genetic system. Many wild strains of P. aurelia contain endosymbionts, but many do not, and even those which do survive perfectly well after the symbionts have been eliminated. There seems to be no particular advantage or disadvantage to a Paramecium in having symbionts. (This is one example of ‘biological untidiness’.) Endosymbionts are also found in other ciliates, for example Euplotes, in some species of which death occurs following removal of the symbionts by antibiotic treatment (Fauré-Fremiet 1952; Heckmann 1975). It may be that here the endosymbionts are really essential for the life of the ciliate, unlike the situation in Paramecium.

Endosymbionts occur in some flagellates, for example in Crithidia oncopelti (Spencer & Cross 1975), and also in Amoeba (Hawkins & Wolstenholme 1967). In one giant amoeba – Pelomyxa palustris, the possibility has been considered that symbionts might play the rôle of mitochondria, which are absent in that species (Whatley 1976). This suggestion can of course only be regarded as an interesting speculation, in the absence of a demonstration that the endosymbionts display the physiological properties of mitochondria.

Since this lecture deals with protozoa, I will not consider endosymbionts in higher organisms. It is however worth pointing out that, as in protozoa, a number
of examples of cytoplasmic inheritance in higher organisms have been shown to be correlated with the presence of bacterium- or virus-like entities in the cytoplasm, e.g. 'incompatibility' in *Culex* (Yen & Barr 1974), 'sex-ratio' in *Drosophila* (Poulson 1963; Oishi & Poulson 1970), and 'CO₂-sensitivity' in *Drosophila* (L'Héritier 1970). Thus our understanding of one type of cytoplasmic heredity owes much to studies on the endosymbionts in *Paramecium*.

**Mitochondria**

Studies on mitochondrial genetics in *Paramecium* were first reported in 1969 (Beale 1969), following much earlier work with other organisms, such as *Neurospora* and above all *Saccharomyces*. In some respects *Paramecium* has advantages for this type of work. The most important is its suitability for microinjection of purified cell fractions by the techniques developed by Koizumi (1974) and Knowles (1974). Using Knowles's technique, it is possible to inject a hundred or more paramecia in a few hours. The value of this technique will become apparent from some work to be described below.

Resistance to certain antibiotics – erythromycin, chloramphenicol, mikamycin – which inhibit protein synthesis in mitochondria (and in bacteria) – had been shown previously in yeast to be due to mutations of mitochondrial genes (as well as of nuclear genes). Cultures of paramecia were therefore exposed to these drugs at appropriate concentrations and drug resistant strains readily obtained after a delay of two weeks or so, at a frequency of approximately 1/10⁸ paramecia (or about 1/10⁶ mitochondria). Preparations of mitochondria were made from the resistant strains and samples injected into drug-sensitive paramecia, which were then placed in culture medium containing antibiotic. After a delay of 5–10 days, a high proportion (sometimes all), of the injected paramecia were seen to have become resistant, due to the replacement of the original sensitive mitochondria by resistant ones derived from the donor strain (Beale et al. 1972).

The microinjection technique has enabled us to carry out experiments bearing on a number of problems of mitochondrial genetics. First, one can show directly that certain characters – e.g. resistance to the drugs mentioned above – are controlled by the mitochondrial genome, presumably mitochondrial DNA. In yeast this could only be done indirectly, by complex experiments involving the demonstration that drug-resistance factors were not usually transmissible through 'petite' yeast cells containing substantial deletions in their mitochondrial DNA (Linnane et al. 1968; Thomas & Wilkie 1968). Secondly the microinjection technique has made it possible to study interspecies variation in *Paramecium*, since in some cases mitochondria can be successfully transferred from one 'species' of *P. aurelia* to another, and diverse nuclear/mitochondrial 'hybrids' constructed.

It has been shown by Cummings (1976) that mitochondrial DNA in the 'transferred' mitochondria maintains its original composition, so far as can be established with the limitation of the techniques available. In studies with restriction
endonucleases, the mitochondrial DNAs from species 1, 5 and 7 were shown to be distinct, and ‘hybrid’ cells contained mitochondrial DNA characteristic of the mitochondrial, not the nuclear, parents.

Nevertheless ‘transferred’ mitochondria have been shown to undergo modifications due to their association with a different nuclear genome. For example, as already mentioned, mitochondria can be readily transferred from species 1 to 7 of *P. aurelia*. The reverse transfer, however, from 7 to 1 has never been successfully carried out. Now if we take mitochondria from ‘hybrid’ cells (sp 1 mitochondria + sp 7 nuclei), they also fail to be transferable back to species 1. Apparently the characteristics of these mitochondria, which as shown above probably contain mitochondrial-DNA like that of the original species 1 paramecia, have been changed by the species 7 nucleus. Thus, interesting interactions between nuclear and mitochondrial genomes can be studied and it is hoped to pursue this type of analysis further.

One can already draw interesting analogies with some other cell systems. For example in higher plants there is the widespread phenomenon of cytoplasmically-inherited male sterility (Edwardson 1970). It can be speculated that some types of male sterility could be due to harmful interactions between mitochondria or other cellular organelles from one strain or species and nuclei of another.

Interspecies transfer of mitochondria in *Paramecium* has also been studied by Tait *et al.* (1976) to show that some mitochondrial proteins, both in the mitochondrial membranes and in the mitochondrial ribosomes, are coded by the mitochondrial genome. As regards the mitochondrial ribosomal proteins, this is not consistent with results of work on yeast and other organisms, from which it has been believed that all the mitochondrial ribosomal proteins are synthesized on cytoplasmic (i.e. not mitochondrial) ribosomes, and hence are usually assumed to be coded by nuclear DNA (review in Schatz & Mason 1974). This requires further study.

So far there has been no clear evidence in *Paramecium* of the occurrence of recombination involving mitochondrial DNA, which has been such an important feature of the yeast system (Netter *et al.* 1974; Dujon *et al.* 1976). Adoutte (1974) has obtained tentative indications of such recombination in *Paramecium*, but his results might also be accounted for by mutation.

It is evident that studies on mitochondrial genetics in *Paramecium* and other protozoa will expand our knowledge in this area of genetics, drawing attention to difference between yeast and other organisms in this matter, and making possible various types of experiment impracticable with yeast, such as those involving the construction of interspecific nuclear/mitochondrial ‘hybrids’.

Before leaving the subject of mitochondria in protozoa, I should like to refer to the kinetoplast of Trypanosomes and related flagellates. This organelle is, at least under certain conditions, situated very close to, but spatially separated from the base of the flagella. The kinetoplast is self-reproducing in the sense that, once lost, it cannot be reconstituted. Recent work (see Simpson 1972; Steinert & van Assel
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1975) has shown that it is part of the large single mitochondrion found in these flagellates. As compared with the DNA in mitochondria generally, however, the kinetoplast DNA is enormous in amount per cell, and shows a most unusual structure. It comprises some $10^4$ interlocking 'minicircles', each of length 0.25–0.75 nm (depending on the species), and in addition usually a proportion of larger (ca. 12 nm) circles, comparable in size to the mitochondrial DNA of other protozoa.

The minicircles are so small as to be able to code for only about one protein or possibly some RNA, and their function is obscure. It is to be expected that much more will soon be known about them. When treated with some dyes the kinetoplasts may be eliminated, and this may have lethal effects on some, but not other, haemoflagellates. Thus the study of these organelles is important both theoretically and practically, in view of the great medical and agricultural importance of some of these protozoa.

Unfortunately no genetic work has yet been done with any member of the Kinetoplastidae, which include parasites belonging to the genera Trypanosoma, Leishmannia and some others, as well as some free-living forms, such as Bodo. No clear evidence for a sexual stage in these protozoa has been demonstrated.

In concluding this account of cytoplasmic DNA-containing structures, I should like to point out that studies with both endosymbionts and mitochondria have not led to any results which are seriously inconsistent with modern genetic theory. Their heredity, like any other, is based on their DNA. This heredity is, of course, 'non-Mendelian' in that it is not controlled by chromosomally located genes. Perhaps it should be considered as a type of prokaryotic heredity in eukaryotic cells.

The fact that genetic studies on both endosymbionts and on mitochondria have been carried out in the same organism (Paramecium) makes it tempting to consider the notion, now widely discussed, that mitochondria have evolved in the distant past from endosymbionts. Space and time do not permit me to go into this question here. However, I would like to draw attention to some differences between contemporary mitochondria and contemporary endosymbionts. As I have indicated above, endosymbionts seem to be governed largely by their own DNA, not by that of their host cell; whereas with mitochondria we have the opposite situation: most mitochondrial characteristics are encoded in the nuclear genome, as has been shown by extensive work with yeast (see Schatz & Mason 1974) and other organisms, and confirmed to a limited extent with Paramecium (Tait 1968, 1970; Knowles & Tait 1972). I will not mention other points of distinction between endosymbionts and mitochondria, since fairness would then also require me to list a number of similarities. Protozoa seem to offer a number of advantages for investigations bearing on questions of the evolution of mitochondria, since so many protozoa contain both endosymbionts, sometimes with included viruses, and, of course, mitochondria.
CELL HEREDITY NOT BASED ON DNA-CONTAINING PARTICLES

Paramecium displays a number of phenomena which may be described as examples of cell heredity, but which are not associated with any visible DNA-containing structures. With a fixed genome, both nuclear and cytoplasmic, the cell is able to function in a number of alternative ways, and these alternative cellular types may be maintained for long periods involving many cell generations, sometimes indefinitely. Here I will consider only one example, that of the surface or immobilization antigens in P. aurelia. A somewhat similar system exists also in Tetrahymena pyriformis (Nanney & Dubert 1960; Nanney 1963).

The genetic basis of the Paramecium antigens was studied by Sonneborn and myself about 25 years ago (for the early literature see Beale 1954). These antigens are proteins covering the pellicle and cilia, and vary in a complex way. They are controlled by a series of genes at different chromosomal loci, each gene coding for a particular antigen. Under different environmental conditions (e.g. temperature), and sometimes even without any environmental change, genes at different loci are called into expression. Usually only genes at one locus are expressed at a given time in a given cell, the remaining antigen-determining genes being ‘unexpressed’, i.e. they have no apparent effect on the phenotype. The control over which genes are expressed, and which are prevented from being expressed, is still not understood. Some early experiments showed that a cytoplasmic factor was concerned, though the cytoplasm was itself also influenced by both genes and environment. Many further details of the system have been described, and are mentioned in various reviews (Beale 1957; Sommerville 1970; Finger 1974).

Here I would just like to stress the idea of variable gene expression. At the present time this notion is widely accepted and is assumed to be the basis, or one possible basis, of cellular differentiation in multicellular organisms. Such a view was formerly not so readily accepted. When describing the Paramecium antigen system I have been rather frequently asked whether some alternative explanation might not be more reasonable. For example it has been suggested that antigen variation might be due to replacement of one kind of bacterial or viral symbiont by another, since Paramecium feeds on other microorganisms. Another suggestion is that antigen variation might be caused by the peeling off of a series of onion-skin-like layers on the cell surface. There is adequate evidence excluding these alternatives and supporting the hypothesis of variable gene expression (Beale 1952, 1954, 1974). The idea that a cell of fixed genotype could show a multiplicity of forms, or be capable of synthesizing a number of alternative proteins, by variation in gene expression, was first clearly demonstrated by work with Paramecium. In the early 1950s the theory of protein synthesis based on transcription from DNA and translation of mRNA, etc., had not been put forward, and the molecular basis of the Paramecium antigen system could not be forecast, though an attempt to do so was made by Delbrück (1949). In the intervening years, concepts
of control mechanisms have been much expanded in work with both eukaryotes and prokaryotes.

This example should be sufficient to indicate the value of protozoa as model organisms for the study of cellular phenomena based on an interplay of nuclear and cytoplasmic determinants. A number of other examples could be given, as mentioned above (p. 16).

**Transplantation experiments in amoeba**

*Amoeba* is another protozoon which has been used for studies of the rôles of nuclear and cytoplasmic determinants. This work has been carried out by transplantation experiments. Many years ago amoebae were subjected to crude operations, whereby a cell was cut into two portions, one containing the nucleus and the other lacking it. From the behaviour of the two portions, some deductions regarding the rôle of the nucleus were made. Later Commandon & de Fonbrune (1939) devised the technique of nuclear transplantation, which has been much used by later workers (Lorch & Danielli 1953; Hawkins & Cole 1965; Hawkins 1973). Evidence has been obtained that some characters are controlled by nuclear factors and others by the cytoplasm, but the precise nature of these factors has not been made clear. It has been suggested by Yudin (1973) that even some of the ‘nuclear’ variation might be of a non-genetic nature. Moreover some of the amoebae used in these experiments are now known to contain endosymbionts (Hawkins & Wolstenholme 1967), the rôle of which has not been established.

The problems in interpreting these results illustrate the need for an ideal model experimental organism, which should like *Amoeba* be convenient for artificial transplantation of nuclear and cytoplasmic constituents, but should also have the capacity for sexual reproduction and be suitable for precise genetic analysis like *Paramecium*.

**Genetics of malaria parasites**

About 8 years ago I started a programme of research to investigate the genetics of malaria parasites. As experimental material we decided for technical reasons to use the rodent parasite – *Plasmodium berghei* – and related species. The motive for this work was partly a practical one, in that malaria is still a very important world health problem, and current methods using anti-malarial drugs are becoming less effective due to the spread of drug-resistant strains of the parasites. I also hoped to make a study of antigenic variation in a parasitic organism along parallel lines to those used previously with *Paramecium* (so far this has not been done).

Considering that malaria parasites require two hosts (a vertebrate one and a mosquito one) to complete their life cycle, and that no *in vitro* culture technique was available (except for limited periods and at certain stages), the project might seem at first sight rather impracticable. Nevertheless my colleagues D. Walliker and R. Carter and some others were prepared to have a go at the task, and
interesting results have been obtained (Walliker et al. 1973, 1975; Beale et al. 1977).

Our work has shown that these organisms exhibit orthodox Mendelian heredity. There is a regular eukaryotic life cycle, with an alternation of haploid and diploid phases. Fertilization and meiosis occur in the mosquito gut. Among the genetic characters studied are electrophoretic variants of enzymes, drug resistance, virulence and antigenic differences. All show normal segregation and recombination. There are no indications that recombination occurs at any stage other than at meiosis—i.e. no evidence for the existence of plasmid-born resistant transfer factors like those known in bacteria. Nor have any examples of cytoplasmic heredity been found so far.

Although this work has not resulted in the discovery of any new genetic principles, it has nevertheless considerably added to our knowledge of malaria parasites. We now know that a single ‘isolate’ or sample of parasites from a single vertebrate host, may consist of a population of genetically diverse clones of parasites, as shown by studies on enzyme variation, using starch gel electrophoresis. In wild populations there are indications both in rodent malaria species (Carter 1973; Beale et al. 1977) and in human malaria (P. falciparum) species (Carter & McGregor 1973), that random assortment of genetic factors takes place in mosquitoes, generating a considerable amount of variation within populations belonging to a given sub-species. Thus although, so far as we know, plasmid transmission of drug-resistance does not occur, there is ample opportunity for drug-resistant genes to spread through a population, recombining with other factors.

Our work on enzyme variation has made it possible to clarify the classification of malaria parasites into species and sub-species, and to show that basically the same polymorphic population of P. falciparum occurs in east and west Africa. It is hoped to extend such studies to southeast Asia and other parts of the world.

Mutants resistant to the drugs pyrimethamine and chloroquine have been obtained in the laboratory by growing strains of rodent malaria parasites under mild drug pressure, most readily by gradually increasing the drug concentration from an initially low level. This is perhaps not surprising since the number of parasites in the blood of a single individual mouse may reach $10^9$ (and in man $10^{12}$), giving ample opportunity for mutations to arise. Once they have arisen, the drug-resistant mutants may be tested for their selective advantage or disadvantage over the original sensitive parasites, even in the absence of further drug pressure. Preliminary experiments of this type have shown, rather alarmingly, that resistance to chloroquine seems to be advantageous over sensitivity, even in the absence of the drug (Rosario et al. 1976). In this connection it is worth recalling that chloroquine resistant strains of P. falciparum, the most important human malaria parasite, are becoming widespread in southeast Asia and South America.

Current practice in malaria treatment seems to be developing along the lines of using more drugs, higher concentrations and mixtures of drugs. This may well
prove counterproductive due to the steady increase in drug-resistant mutants. Further progress in devising methods for dealing with malaria parasites may be better served by obtaining more basic knowledge, especially of genetics, about the parasites, than by searching for new drugs.

**BEHAVIOURAL MUTANTS OF PARAMECIUM**

As a last example of genetic work on protozoa I should like to refer to the recent studies of Kung and his associates (Kung *et al.* 1975; Kung 1976) on behavioural mutants of *Paramecium*. This type of work shows that *Paramecium* may prove to be a useful model experimental organism for the study of certain neurophysiological problems applicable to higher organisms.

'Behaviour' in *Paramecium* is rather simple and predictable, under standard conditions. The organism normally moves forward with a spiral motion, due to the coordinated beating of its cilia. When an unfavourable obstacle or chemical is encountered, paramecia display an 'avoiding reaction'. As described long ago (1906) by Jennings (see Kung 1975) this consists of a temporary backwards movement, due to reversal of the ciliary beat, and then a resumption of normal forward swimming, usually in a direction at an angle to the original. In this way there is hope that an obstacle would be by-passed. The ciliary reversal has been shown to be correlated with a depolarization of the surface membrane, that is by an alteration of some component of the latter controlling maintenance of a negative change in the interior of the cell.

A large number of mutants affecting the response of paramecia to harmful stimuli have been obtained. These mutants, which all show Mendelian inheritance, form three groups, controlled by genes at three loci. One class of mutants is denoted 'pawn'. Like pawns in chess these protozoan mutants are incapable of reversing their movement, and hence display somewhat suicidal tendencies when confronted with a dangerous situation ahead. Another class of mutant is denoted 'paranoiac', and animals of this type respond to adverse stimuli by moving backwards for prolonged periods.

By inserting a microelectrode into the interior of a paramecium, and recording changes in electric potentials, Kung and his colleagues have compared the responses of various mutant and wild type organisms to adverse stimuli. This makes it possible to classify the mutants in regard to their ability to show depolarization and repolarization of their surface membranes. At present it is not known what components of the membranes, whether protein or some other, are involved in these reactions. but it is hoped that future work will result in an increase in our knowledge of this problem. Here genetics is being used as a method of ‘dissection’ of a complex system, a method which has been previously used with great success in *Neurospora*, *E. coli* and other organisms to unravel details of intermediary metabolism.

Assuming that the response of the surface membranes of ciliate protozoa to
stimuli is analogous to that of nerve and other cells in higher organisms, the use of behavioural mutants of Paramecium may eventually contribute to our further understanding of neurophysiology; though notwithstanding the terminology used to depict some of the mutants, the psychiatric benefits of the work are somewhat less promising. As discussion of these matters may raise questions about the psychological complexity, or ability to manifest consciousness, of protozoa, this seems an appropriate moment to end the lecture.

CONCLUSION

I hope what I have said will be sufficient to draw attention to the great value of protozoa as experimental organisms for the study of certain genetic phenomena, especially those dealing with different types of cytoplasmic heredity, some based on DNA-containing particles, some not; and also of various other interesting and little studied problems, such as the genetic basis of neurophysiological behaviour; and finally to show the need for genetic work on medically important parasitic protozoa, such as malaria parasites, trypanosomes and others.

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