Perspectives

Anecdotal, Historical And Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Whatever Happened To Paramecium Genetics?

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In the period between 1945 and 1965 the genetics of Paramecium enjoyed its heyday. All introductory college texts on genetics had a section on Paramecium. Stadler et al. (1965) had the most extensive coverage. Topics considered were the life cycle, infectious heredity (killers), stable states of gene expression (serotypes), the ability of preexisting structure to control the development of new structure (cortical inheritance), and inheritance due to macronuclear differentiation (mating types). At a more advanced level, The Genetics of Microorganisms (Catcheside 1951) had a whole chapter on protozoan genetics (almost all on Paramecium), alongside chapters on viral genetics and bacterial genetics.

A perusal of many of today's popular introductory textbooks of genetics reveals that the vast majority, such as Griffiths et al. (1993) and Russell (1994) are completely free of all references to Paramecium. Only an occasional text such as Klug and Cummings (1994) or Tamarin (1994) presents some of the findings on Paramecium. At the more advanced level, Genes V (Lewin 1994) has only one reference to Paramecium and, alas, it is wrong. In the same book a discussion of deviations from the universal genetic code in ciliates, first discovered in Paramecium, mentions only Tetrahymena and Euplotes.

The facts presented in those early times haven't changed, and, indeed, our knowledge of most of the subjects has been advanced considerably since then. Nevertheless, the work on Paramecium appears either to have been judged inconsequential or else has just been forgotten. This paper is devoted to the question of what happened. Is the work truly inconsequential? Has only a loss of memory occurred? And if the work was forgotten, then why?

In our search for answers to these questions we go back in time. In the early days of T. H. Morgan in the 1920s, there was somewhat of a battle going on between the "Mendelian-Morgan geneticists" and the "physiologists." The geneticists had established the chromosome as the basis of heredity and the genes as the units of inheritance. The physiologists maintained that the genes being studied by geneticists all affected rather trivial and superficial characters, which were capable of undergoing mutation. The really important characteristics such as membrane permeability, cell mobility, and cell division were invariant and determined by the cytoplasm, not the genes on chromosomes. Mutations affecting such fundamental traits, they argued, would invariably lead to cell death and were, therefore, inaccessible to the science of genetics. H. S. Jennings at Johns Hopkins felt that the argument was worth attention and that the protozoa might well be able to contribute to the answer. For accounts of Jennings, see Sonneborn (1975b) and Crow (1987). Protozoan genetics was a flourishing science at that time, and Jennings' (1929) review of the subject had 259 references. While Paramecium was not the only game in town in 1929, it was surely a major one. Moreover, some of that work didn't seem to fit with the notion that all heredity could be explained by assorting genes on chromosomes.

Since that time, much has been learned about the genetics of Paramecium, primarily owing to the work of Sonneborn and his students. Sonneborn (Preer 1996) was a giant figure in protozoan genetics, laying the basis for studies on virtually all the subjects now present in the modern work on ciliates. With the advent of Watson and Crick's double helix, the focus of research in genetics changed, and the earlier arguments mentioned above have been largely forgotten. We will go back and review the findings that seem pertinent, bringing each topic up to date. Perhaps in this way we will be able to understand what has happened and judge better what the significance of each area is.

The macronucleus: Paramecium, like most other ciliates, has one or more diploid micronuclei and a polyploid macronucleus. The polyploid macronucleus is the metabolically active nucleus, while the genes of the micronucleus are largely unexpressed. At conjugation the micronuclei undergo meiosis, while the old macronucleus starts to degenerate. One of the meiotic products

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Genetics 145: 217-225 (February, 1997)
now divides once to produce a stationary and a migratory haploid nucleus. After migratory nuclei are exchanged, the migratory and stationary nuclei unite to produce a diploid syncaryon, which divides mitotically twice to produce nuclei that develop into new micronuclei and macronuclei. Autogamy is identical except that the process occurs in a single cell, the migratory and stationary nuclei formed in each cell simply fusing to produce the syncaryon. It was always obvious that a knowledge of the fundamental structure of the macronucleus was necessary to understand the genetics of Paramecium. However, the techniques suitable for attacking this problem were not available until fairly recently. It has been known for a very long time that extensive amplification occurs at macronuclear formation, ploidy levels going from diploid to 1000 or more copies. We know now that, in addition to amplification, extensive rearrangements in the DNA also occur.

Two major types of DNA rearrangements are found in ciliates (see the review by Prescott 1994). In the first kind, chromosomes are simply broken (usually with concomitant loss of a few bases), and telomeres are added to the broken ends. The breaks occur at a conserved 15 base pair chromosome breakage sequence in Tetrahymena, where it is estimated that several thousand such breaks occur at the formation of a new macronucleus (Yao 1988). The size of the resulting macronuclear chromosomes varies with the ciliate species, reaching its most extreme case in the hypotrichous ciliates, where the rule is one macronuclear chromosome for one gene. More recently, information is also becoming available about such breaks in Paramecium tetraurelia (Preer and Preer 1979; Forney and Blackburn 1988; Phan et al. 1988; Caron 1992), where each of the many micronuclear chromosomes is broken into an average of about seven new macronuclear chromosomes with an average size of 300–600 kb. We know that there is often variation in the way these events occur and that, once formed, the resulting variations can be constant throughout the life of a given macronucleus (Amar 1994). Telomeres consist of C4A2 repeats in Tetrahymena and a mixture of C4A2 and C3A3 repeats in Paramecium. Telomerase, the enzyme responsible for telomere addition, is a ribonucleoprotein, with the RNA component containing a complement of C4A2. Surprisingly, the RNA is encoded by a gene that contains only the complement of C4A2 in both Tetrahymena and Paramecium (McCormick-Graham and Romero 1994).

In the second kind of chromosome breakage, chromosomes undergo internal deletion. The deleted sections, which never get into the macronucleus and are lost, vary in size from about 20 to several hundred bases and are called internal eliminated sequences (IESs). Such sequences are very numerous and are often found within the coding region of a single gene (Steele et al. 1994). The gene for surface protein A in Paramecium contains seven IESs within the coding region of the gene and two more just upstream. In Paramecium and Euplotes, IESs are bounded by the dinucleotide, TA, and a short consensus sequence within the ends of the IESs has been characterized (Klobutcher and Herrick 1997). The deletions are made with perfect precision, and the resulting macronuclear genes have intact coding regions. Remarkably, it has been found (see review in Prescott 1994) that in some ciliates the portions of the genes between the eliminated pieces are in a scrambled order in the micronucleus, and during macronuclear formation the sections destined for the macronucleus are miraculously recombined to give properly ordered functional genes. Some of these internal eliminated sequences have homology to transposon-related sequences found in the micronuclei of ciliates (Klobutcher and Herrick 1995). It is interesting that in ciliates the transposon-like elements are eliminated during macronuclear formation. The transposon-related sequences have also been shown to show sequence homology with well-known transposons found in a variety of organisms (Doak et al. 1993).

The macronucleus is a particularly suitable place to study the many kinds of variation that can occur in DNA.

**Amitosis:** When cells divide, they do so by mitosis, which ensures that the chromosomes of the parent are precisely reproduced in the daughter cells. When the macronuclei of ciliates divide, however, there is no mitosis and the macronuclei simply pinch into two by amitosis. This process seems to be the only true case of amitosis known. The question of how genic balance is maintained during many cycles of amitosis has never been answered. In the absence of mitosis, random distribution of chromosomes at cell division should lead to the drift in the numbers of the different kinds of chromosomes and result in imbalances. If divisions continued long enough, some chromosomes would even be lost altogether. Such drift does occur in Tetrahymena in the case of chromosomes bearing alternative alleles, for heterozygous lines show allelic segregation during continued fissions, eventually producing lines pure for one or the other allele. Although single alleles are segregated out into pure lines, genic balance is maintained permanently among genes at different loci. Evidence obtained in Tetrahymena shows that in lieu of mitosis, some other mechanism must operate in ciliates to maintain balance (see Preer and Preer 1979). It is difficult to conceive of any simple mechanism that might produce proper balance. According to one hypothesis (Preer and Preer 1979; Brunk 1986), each kind of macronuclear chromosome can sense its own copy number (much like different plasmids in a bacterium) and adjust its replication in such a way that it maintains a constant level. This is an old problem that cries for a solution. The existence of unique sites on mac-
rnonuclear chromosomes that determine copy number is an exciting possibility.

Caryonidal inheritance: Soon after Tracy Sonneborn discovered mating types in Paramecium, he began a serious study of the genetics of Paramecium. He first looked at the inheritance of the newly discovered mating types, quickly demonstrated simple Mendelism, and confirmed the essential nuclear events at cell division, conjugation, and autogamy. As already noted, new macronuclei and micronuclei are derived from a single diploid micronucleus. These are segregated to separate cells at the next cell division. Sonneborn called these lines "caryonides" and found that they were often of different mating type, even though both the nuclei were derived from a single diploid nucleus by mitosis. Caryonidal inheritance was defined as the inheritance of characteristic differences that arise at the time of macronuclear formation and remain constant throughout subsequent vegetative fissions. We now know that variations in the organization of macronuclear DNA often arise when new macronuclei develop from micronuclei (Amar 1994). Such variations are also inherited caryonidally, and it is likely that caryonidal inheritance of mating type has a similar explanation (Orias 1981). DNA processing, that is, the production of variations in DNA, is well known in many organisms (Ascaris, scale insects, development of the immune system in vertebrates). Macronuclear development gives us a unique system in which to study the phenomenon of DNA processing.

Transformation: Godiska et al. (1987) showed that the macronucleus of Paramecium can be transformed by injecting DNA. The disadvantage of having to inject cells individually is more than offset by very high transformation frequencies (nearly 100% in the hands of some investigators). Telomeres are added directly to the terminal bases of the ends of the injected DNA (Gilley et al. 1988). Unlike Tetrahymena, Paramecium can apparently add telomeres to any piece of DNA from any source and then provide for its reproduction. Moreover, injected genes derived from Paramecium are transcribed and translated. The DNA is usually replicated at the same level at which it is injected (Kim et al. 1992) and finally is lost at the next autogamy or conjugation. In Tetrahymena, fragments of DNA do not acquire telomeres, but suitable vectors have been produced, and integration of genes into preexisting chromosomes occurs. Thus, transformation produces a macronucleus consisting of a few transformed macronuclear chromosomes mixed with a large number of untransformed. At subsequent vegetative fissions, these types segregate out into pure types and make it possible to carry out gene replacement. These new techniques are proving to be powerful additions to our ability to study the molecular genetics of ciliates.

Macronuclear inheritance: Perhaps the most unusual and intriguing phenomenon in ciliate genetics was discovered many years ago when Sonneborn was first studying the inheritance of mating type. Although mating type in most strains of Paramecium showed caryonidal inheritance, as described above, in one group of species the caryonidal inheritance was found to be modified by a very strong tendency for the mating type of each caryonide to be like that of its cytoplasmic parent. In other words, cytoplasmic inheritance was involved. Sonneborn, however, was able to show by an ingenious experiment, using conjugation, cytoplasmic exchange, and regeneration of fragments of the old macronucleus, that the genetic determinants lay in the macronuclei, not in the cytoplasm. Using genetic markers, he was able to recover and distinguish lines whose macronuclei were derived from normally produced syncarya and lines whose macronuclei were derived from regenerating fragments of the old macronucleus. He found that determination of macronuclei occurs only during new macronuclear formation and is usually the same type as the type of the old macronucleus. He concluded that the macronucleus determines the cytoplasm, and the cytoplasm, in turn, determines the type of newly forming macronuclei. Thus, genetic information is transferred by way of the cytoplasm from the old macronucleus to the new macronucleus at conjugation and autogamy. This pattern of inheritance has subsequently been called macronuclear inheritance. Later discoveries have led to considerable advances in our knowledge of the situation.

Most of these advances began with the discovery of a puzzling mutant discovered by Epstein and Forney (1984). This mutant appeared in a screen for mutants that were unable to express surface antigen A in stock 51 of Paramecium tetraurelia. Although most of the mutants proved to be Mendelian, one, d48, showed a cytoplasmic pattern of inheritance. Since the A gene had been isolated, it was possible to ascertain that the chromosome bearing the A gene, which was near the end of a macronuclear chromosome, had been deleted at a point just upstream of the A gene, eliminating the entire gene from the macronucleus. The genetic results, however, were consistent only with the conclusion that the macronucleus still contained the complete A gene [later proved directly with the isolation of micronuclei in Paramecium (Preer et al. 1992)]. Thus, d48 was a defect in DNA processing. Soon after this discovery, Harumoto (1986) found that d48 could be "rescued" (converted to wild type permanently) by injecting into its macronucleus macronuclear material from wild-type cells. This surprising discovery was soon followed by the observation that the injection of the whole of the cloned A gene, or even a small portion of the A gene, sufficed for rescue (Kim et al. 1994; You et al. 1994). There is more than one effective region in the A gene, and the longer the region, the more effective it is. It was concluded that the genetic defect in d48 that leads to its apparent cytoplasmic inheritance is only the lack of the A gene in the old macronucleus. If this
conclusion is correct, then one should be able to create d48 mutations at will as follows. The micronucleus of a mutant strain containing a micronuclear deletion for the A gene is removed by micromanipulation and then replaced with a micronucleus from wild type. When this experiment was carried out (Kobayashi and Koizumi 1988), it was found that the new lines were stable through subsequent autogamies and were indistinguishable from d48. This dramatic result confirms that d48 is not inherited as a simple cytoplasmic mutant, but is a micronuclear DNA processing mutation.

The same phenomenon has been shown for the serotype B gene. A d48-like mutation defective in serotype B was constructed by removing the micronuclei from a mutant deficient in its micronucleus for the B gene and transplanting in new micronuclei from wild type (Scott et al. 1994). The B gene cannot rescue d48, and A cannot rescue the d48-like no-B mutation, nor will any other DNA serve the purpose. Specificity is complete. No complementary RNAs appear to be produced. The explanation for micronuclear inheritance is totally unknown. One suggestion is that normally, when new micronuclei are formed, small bits of DNA leave the A gene in the old degenerating micronucleus, pass through the cytoplasm, enter the newly forming macronuclei, and serve as an essential element in DNA processing of the regions in and adjacent to the A gene.

Macronuclear inheritance is exhibited not only by mating type and d48 and d48-like mutants, but also by a trichocyst non-discharge mutant studied by Sonneborn and Schneller (1979) and by reversion of a paranoiac behavioral mutant by Rudman and Preer (1996). Mating type has recently been associated with rearrangements in Paramecium (Meyer and Keller 1996). The generality of micronuclear inheritance is further revealed by a remarkable observation of Meyer (1992). He injected very high amounts of various clones of wild-type DNA into wild-type cells. Surprisingly, he found that offspring of such injected cells carry deletions in their DNA. These occur in the vicinity of the chromosomal location of the sites from which the injected DNA was taken. Moreover, further analysis of these clones shows that the deletions exhibit macronuclear inheritance. These experiments reinforce the conclusion that the DNA of the old macronucleus can influence DNA processing during new macronuclear formation. Elucidation of these remarkable events at the molecular level will give us new insights into the mechanisms that control DNA cutting and splicing.

Killers: Sonneborn found that, although Mendelian genetics plays the same role in Paramecium as it does in other organisms, it often fails to provide satisfactory explanations for the phenomena he encountered. Not only did cyanoidal inheritance, amitosis, and the life cycle present problems, but every other trait that he examined seemed to show some sort of non-Mendelian behavior. This was true of antigenic variation, cell shape, and, most dramatically, the inheritance of “killer” paramecia. Killer paramecia constituted the first example of cytoplasmic inheritance in animals, although many cases had been found in plants. Killers liberated a toxin into the medium that killed non-killer (sensitive) paramecia. Crosses showed that the trait was dependent upon a cytoplasmic factor which Sonneborn designated “kappa.” Kappa, in turn, depended upon the presence of the Mendelian gene K. Kappa also rendered the cells in which it was found resistant to the toxin.

At about this time Ephrussi was beginning his classic work on yeast that eventually led to our understanding of mitochondrial genetics. S. Spiegelman also found non-Mendelian phenomena in his studies on adaptive enzymes in yeast. When Sonneborn viewed his own findings on Paramecium in the light of these other discoveries, the “plasmagene theory” (Sonneborn 1947) was proposed. It was hypothesized that genes could produce messengers with the capacity for self-reproduction. The work on kappa constituted the underpinnings of the plasmagene theory. Later it became increasingly evident that kappa was a symbiotic bacterium, not a self-reproducing gene product. Moreover, the inheritance of enzymatic adaptation in Escherichia coli, which closely resembled that in yeast, was explained in terms of metabolic steady states (Novick and Weiner 1957). Plasmagens were gone even before they had become well known. Moreover, the other phenomena that had led Sonneborn to the proposal, as we will see, eventually turned out to have different bases.

Subsequent work on kappa revealed many species of bacterial symbionts present in Paramecium (see reviews in Preer and Preer 1984; Pond et al. 1989). Each of the symbionts has a restricted cellular location, a few in the micronuclei, some in macronuclei, most in cytoplasm. Some have flagella, some have a thick cell wall. None are capable of culture free of Paramecium. Different strains produce different toxins, each resulting in a different prelethal effect on sensitive paramecia. Some of the toxins are found in the medium in which killers live; some are active only during cell-to-cell contact at conjugation; some produce no toxins at all. The production of toxins has been related to the presence in the symbionts of plasmids in some cases, to defective phages in others. The toxins appear to be insoluble substances and in many cases are found associated with refractile (R bodies) that develop within kappa, minute, tightly wound rolls of proteinaceous ribbons that can unroll and reroll in a fraction of a second in response to certain environmental stimuli. The gene for one type of R body has been found on one of the plasmids found in kappa and has been cloned into E. coli, where it is expressed, thereby making E. coli an R body producer (but not a killer). Each killer strain of Paramecium is resistant to the toxin it produces, and most strains of Paramecium that have been freed of kappa are sensitive.
to the toxin produced by that strain. The function of
the K gene, how the toxins kill sensitive paramecia,
and the mechanism by which kappa is able to confer
resistance on its host present intriguing but unanswered
questions. The significance of the kappa story is that
free-living organisms can enter cells and become inte-
gral parts of the workings of the hosts. Even mitochon-
dria and chloroplasts are presumed to have arisen in
this way. The studies on Paramecium symbions tell us
that the borderline between heredity and parasitism
can become very blurred.

Serotypes: SONNEBORN found that every clone of
Paramecium has a single major antigenic protein on its
surface, designated A, B, C, etc. (reviewed in FREER
1986; CARON and MEYER 1989). The serotypes are mutually
exclusive, for only a single surface antigen was found on a given cell at a time. The clones are designated
serotype A, serotype B, serotype C, etc., and a single genotype can express approximately a dozen serotypes. Although each type is expressed better under one set of environmental conditions than another, most types may be stabilized under a single set of environ-
mental conditions. SONNEBORN was able to find a single set of conditions under which most serotypes will repro-
duce true to type indefinitely and therefore concluded that the serotypes were inherited. Crosses between any
two serotypes under constant conditions reveal cyto-
plasmic inheritance. Examination of a number of differ-
ent natural strains shows that there are serologically distinguishable differences between the A serotype in each strain, differences between the B serotypes, etc. Moreover, the between-strain differences are inherited as simple Mendelian factors. The conclusion that SON-
NEBORN reached is that there is a series of unlinked genes that are expressed in a mutually exclusive fashion depending upon the cytoplasmic state, and that the cytoplasmic state is subject to environmental influence.

At first SONNEBORN interpreted the results on sero-
types in terms of the plasmagene theory. At a meeting
in Paris on biological units endowed with genetic contin-
uity, DELBRÜCK (1948) suggested that such stable states of gene expression might be explained entirely
by a series of mutually exclusive reactions that occurred
during antigen synthesis. Although the details of this
suggestion were not correct, he was right in pointing
out that diverse hereditary states don’t necessarily ema-
nate from self-reproducing particles, but might be pro-
duced by competing chemical reactions that affect gene
expression. This general conclusion was quickly ac-
cepted as the most probable explanation for serotype
inheritance.

Today the protein antigens, the mRNAs, and the
genes have all been isolated (FORNEY et al. 1983; RUD-
MAN et al. 1991). Although regulation is usually at the
level of mRNA, in some serotype genes it is also regu-
lated posttranscriptionally (GILLEY et al. 1990). The
region starting approximately 200 bp upstream of the
start of translation and extending to the start of trans-
lation is necessary for expression (MARTIN et al.1994;
LEECK and FORNEY 1996). Most of the region is not
transcribed and is thought to contain the promoter.
LEECK and FORNEY prepared a hybrid plasmid with the
upstream nontranscribed region of the B gene ligated
to the whole of the transcribed portion of the A gene
(including the entire A protein coding sequence).
They then injected various combinations of this hybrid
and complete A and B genes into a strain containing
Mendelian deletions for both A and B. These co-trans-
formed cells were also subjected to different environ-
ments affecting serotype expression (A is favored by
high temperature, B by low.) The hybrid antigen gene
acted exactly like the complete A gene itself, that is,
the putative B promoter acts exactly like the putative
A promoter in its response to the environment. More-
over, regulation was at the level of transcription. It was
concluded that the upstream nontranscribed region
of the gene, while necessary for transcription, is not
involved in the mutually exclusive expression seen
among serotypes. Mutual exclusion is controlled by
the transcribed region of the gene. These results are
consistent with hypotheses that assume stable antigen
expression involving feedback of immobilization anti-
gens or their mRNA (FINGER et al. 1995). Serotype
variation in Paramecium tells us that highly stable
states of genetic expression can develop, leading to
cellular differentiations that can be maintained for
hundreds of cell generations. Such stable states appear
to operate at the level of transcriptional control and
involve feedback loops. Thus, the process of obtaining
an understanding of this phenomenon at the molecu-
lar level is in progress.

Cytotaxis: By the mid-1950s after SONNEBORN had
investigated the inheritance of mating type, killers, and
serotypes, he decided to push his research in a different
direction. The genetic aspects of the kappa story
seemed completed. Although the molecular mecha-
nisms responsible for serotype variation were not
known, it appeared that the serotypes were cases of
stable changes in gene expression, and the techniques
available to understand the mechanisms of such phe-
nomena were also not available at that time. So he de-
cided to turn his attention to cell shape and the nature
of the cellular cortex in Paramecium. When paramecia
conjugate, they do not always separate, and double ani-
mal parts are sometimes formed that reproduce true to type as
double animals. SONNEBORN found that by treating
cells with antiserum he could induce double animals at
will. He crossed single animals with double animals,
and in an ingenious set of experiments he showed that
the genetic basis lay not in the micronuclei, not in the
macronucleus, and not in the fluid cytoplasm. The only
candidate left was the cortical structure itself. In coopera-
tion with J. BEISSON, he found that even a portion of
the cortex could become rearranged and act as its own
genetic element in inheritance. The influence of preexisting structure on the development of new structure he called cytotaxis. The ciliate cortex and its development has since been extensively studied. It has been suggested that cytotaxis is a fundamental process in cellular development and applies to structures found in all organisms. Microtubule organizing centers and cell membranes are candidates. For a detailed discussion of these problems the reader is referred to FRANKEI (1989) and GRIMES and AUFDERHEIDE (1991).

Behavioral genetics: As a graduate student, CHING KUNG read H. S. JENNINGS' Behavior of Lower Organisms, a work better known to psychologists than biologists. He became interested in the behavior of Paramecium. Working as a postdoctoral in SONNEBORN's laboratory in the late 1960s, he developed techniques that enabled him to isolate large numbers of behavioral mutants. Analysis of these mutants soon led to the conclusion that they were largely mutations that affected the electrophysiological properties of the cell membranes in Paramecium by affecting the constitution of membrane channels for Ca\(^{2+}\) and other ions. One of these behavioral mutations was found to be in the gene coding for calmodulin. The use of mutants of Paramecium in studying channels presents an unprecedented opportunity, for simple mutants that affect channel function are not available in higher organisms. KUNG has made major contributions to our knowledge of the electrophysiology of membrane transport. More recently he has found that injection of whole cell DNA from wild type into the mutants can restore normal behavior. Injection of DNA prepared from libraries of whole cell DNA also works. Taking advantage of these observations, he and coworkers have recently been able to screen a library using this technique and to isolate the gene that may encode a specific membrane ion channel protein (W. J. HAYNES, Y. SAIMI, and C. KUNG, personal communication). The identification of channel proteins has proved difficult in work on higher organisms. Significant advances in our understanding of membrane ion channel proteins in Paramecium now seem certain.

The life cycle: After conjugation, paramecia typically undergo stages of immaturity, maturity, and senescence. Each of these stages can last as many as several hundred fissions, depending upon the species. Immature cells, while vigorous, cannot mate. Mature cells are vigorous and mate readily. During senescence fission rate and viability both decline, and unless conjugation or autogamy occurs the cells die. Another feature of the life cycle is that species that undergo autogamy will not do so unless a sufficient number of fissions has elapsed since the last autogamy or conjugation. These slow progressive changes seen in the stages of the life cycle were considered to be subjects for genetic analysis by the early workers in the time of JENNINGS and SONNEBORN (see PREER 1968 for a short review of the early work). Some studies have occurred since those early days and a few are discussed below.

SIEGEL (1961) showed that the genes that determine specificity of mating type are repressed during the immature period in \textit{P. bursaria} and that these genes become active sequentially as the cells enter the period of maturity. HAGA and HIWATASHI (1981) isolated a protein from immature \textit{P. caudatum} called immaturin that is able to transform mature cells into immature cells when injected. It is likely that passage from immaturity to maturity involves changes in stable states of gene repression and gene activation. Immaturin appears to be a regulatory protein. The way is now open to find the gene for immaturin. Perhaps the time has now come to make progress on the molecular biology of immaturity and maturity.

Two recent studies on Paramecium shed light on the problem of aging. AUFDERHEIDE (1987), working on Paramecium, has shown us where the primary site of aging is located. By transferring cytoplasm between young and aging cells by microinjection and by doing the same for nuclear material, he was able to show that the primary cause for aging is located within the macronucleus, not within the cytoplasm. In the field of aging, where almost nothing seems certain, this is a solid finding as well as a promising beginning for further studies. Another interesting study is a test of the theory that aging stems from changes in telomere length (GILLEY and BLACKBURN 1994). They established that senescence is not due to changes in telomere length in Paramecium, for telomere length does not vary significantly during the life cycle. They also noted a great decrease in the size of macronuclear chromosomes during aging.

Nevertheless, the way that Paramecium counts its fissions, what controls the programmed changes in gene expression leading to maturity, and the nature of senescence are not much better understood today than when these fascinating problems were first encountered.

So what did happen to the genetics of Paramecium? We conclude that the facts about Paramecium genetics that we knew in its period of high visibility many years ago are still valid today. We also have reviewed what we consider to be some fascinating new advances in Paramecium genetics as well as some very bright opportunities for future discoveries. We proceed by considering why Paramecium has become, in the words of CHING KUNG, an "endangered genetic species."

Possibility no. 1: One of my colleagues, presumably with tongue in cheek, suggested that autogamy is just too complicated for geneticists to understand. Having seen the blank looks on the faces of many non-Paramecium geneticists at the mention of autogamy, I have concluded that this possibility should be taken seriously. During autogamy all but one of the products of a typical meiosis are lost, and the one remaining haploid nucleus divides, the two nuclei recombine to give a homozygous
diploid nucleus, and this then divides mitotically to produce the new micronuclei and macronuclei. On reflection, I just don't believe that geneticists who seem to be capable of understanding meiosis, recombination, interference, and sex determination in Drosophila are unable to comprehend autogamy. This possibility must be rejected.

**Possibility no. 2:** Paramecium is too difficult to culture. There is a little more truth to this possibility. It may, indeed, account for the fact that Tetrahymena appears to be replacing Paramecium in ciliate laboratories, for Tetrahymena is quite happy with a solution of just Difco protease peptone. Nevertheless, workers in the 1800s made no such complaints, and judging from the smells and boiling pots I sense as I go past Drosophila kitchens, I cannot give much credence to this possibility.

**Possibility no. 3:** With the advent of molecular genetics, there are too many really important things to cover, and there isn't any room for Paramecium genetics any more. I don't think so. The few pages that are included in the present essay have covered far more about Paramecium than need be covered in a student's introduction to modern genetics and would scarcely increase the thickness of such books.

**Possibility no. 4:** Paramecium is just too queer, and nothing that happens in Paramecium is relevant to what happens in other organisms. Well, this isn't everyone's opinion. I can still remember the administrator of my NIH study section in Washington noting that such and such projects on various weird organisms were especially valuable, for they provided much-needed evolutionary diversity to the NIH-sponsored research programs. Of course, I don't know how many people believe that Paramecium is too queer. I am not one of them. SONNEBORN always quoted BATESON in saying, "Treasure your exceptions." They often provide the key to the usual.

**Possibility no. 5:** Funding for research on ciliates has not been available. This possibility has certainly been important in Great Britain, where the decision was made many years ago to put research funds for biology only into areas of clear economic importance. The ciliate workers there have left for other fields of research, several to Plasmodium, a difficult organism, indeed, in which to study genetics. In this country, it is a matter of attacks from various sources, the prevailing view still seems to be that the advance of science is best served by supporting all kinds of basic research. Most panels that influence funding consist of workers on the currently popular organisms, and hence might be suspected of bias. Nevertheless, I do not believe that the proposals for research on Paramecium that have been rejected are any better than the rejected proposals for work on organisms popular at the present time such as Drosophila, yeast, worms, etc.

**Possibility no. 6:** Most of SONNEBORN's students later turned to studies on other organisms. For example, DAVE NANNEY left Paramecium for Tetrahymena, RICHARD SIEGEL left Paramecium for Drosophila, MYRON LEVINE and DAVID SKAAR left Paramecium for bacteria. Now, with two or three laboratories in Europe and not many more in the United States, Japan seems to be the only place where Paramecium is not in danger of disappearing altogether. Perhaps SONNEBORN so dominated the field that others left to prevent being smothered; perhaps they just wanted to spread his gospel to other fields. NANNEY did just that with Tetrahymena, laying the basis for all the current genetic work on that organism (see NANNEY 1980). Perhaps others thought that Paramecium was just too queer.

**Possibility no. 7:** SONNEBORN is not around any more. There is a lot to this possibility. TRACY was a giant among experimentalists. The richness of the knowledge he gave us about the life cycle of Paramecium and how to exploit this knowledge with useful genetic techniques was truly amazing. Moreover, TRACY thought deeply about his research and explored the relevance of all our knowledge of Paramecium to the broad problems in biology of his day. His enthusiasm knew no bounds, and he had to tell everyone about it. He did it by attending all meetings, participating in the workings of many societies, becoming president of the Genetics Society of America, of the American Society of Naturalists, of the American Institute of Biological Societies, participating in symposia without end, becoming a Sigma Xi Lecturer, and giving innumerable named lectures. Many graduate students and postdoctorals from around the world came to his laboratory in Bloomington, Indiana, the Mecca for ciliate studies. Moreover, his enthusiasm was infectious. Undergraduates and graduates alike in his classes were found to have cultures of the organisms he happened to be lecturing about surreptitiously hidden around their rooms. Students could hardly keep from following him from his lectures back to his laboratory to find out the result of some current experiment he had told them about. He is irreplaceable.

**Possibility no. 8:** Paramecium is just irrelevant today. This just has to be wrong, for where could one find an organism so full of possibilities? Cells are infected with a rich flora of all kinds of symbiotic microorganisms that will only grow in Paramecium, many with plasmids or phages that are involved in the killing and also the resistance of the hosts to the toxins they produce. Some traits are due to mitochondria (BEALE 1969). Gene activity or gene inactivity is passed on to the progeny in the case of surface antigens. There is one set of mating types determined by a biological clock that regularly switches from one type to the other every morning and every evening (BARNETT 1966). The arrangement of surface structures is inherited, but how is not known. Macronuclei pass on many of their characteristics to new macronuclei, by an unknown and mysterious mechanism. A simple gene sequencing demonstrates a varia-
tion in the “universal” genetic code (Caron and Meyer 1985; Preer et al. 1985). All injected DNA multiples, and new telomeres attach to any sequence. It is an organism that continues to offer intriguing puzzles to be solved. Almost every natural character leads to some kind of inheritance unknown elsewhere. I don’t think Paramecium is irrelevant today. But then I still work on Paramecium!

LITERATURE CITED


Preer, J. R., Jr., and L. B. Preer, 1984 Endosymbionts of Protozoa,


