NATIONAL ACADEMY OF SCIENCES

TRACY MORTON SONNEBORN

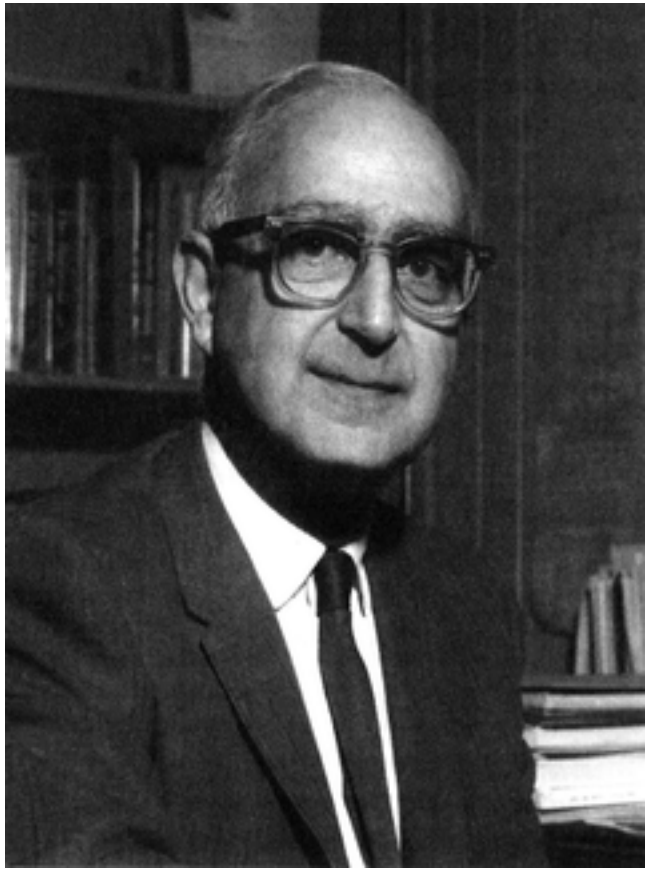
1905—1981

A Biographical Memoir by

JOHN R. PREER, JR.

Biographical Memoir

COPYRIGHT 1996
NATIONAL ACADEMIES PRESS
WASHINGTON D.C.



Photograph by William Dellenback, Bloomington, Indiana

T M Sonneborn

TRACY MORTON SONNEBORN

October 19, 1905–January 26, 1981

BY JOHN R. PREER, JR.

With pipettes, culture vessels, a low- and a high-power microscope, and a few collections of water from nearby ponds and streams, Tracy Sonneborn worked with species of the *Paramecium aurelia* group and learned more about the basic biology of protozoa than anyone else ever has. He discovered mating types in *Paramecium*, thereby advancing biological studies on the protozoa by a quantum leap. He demonstrated simple Mendelism and established the behavior of genes, nuclei, and cytoplasm in the complex processes of the life cycle. He showed that the uniparental nuclear reorganization that occurs periodically in many paramecia is the sexual process of autogamy, not the asexual process of endomixis as originally thought.

He discovered macronuclear regeneration and cytoplasmic exchange, both invaluable for genetic analysis. He demonstrated caryonidal inheritance, showing that individual ciliate macronuclei, although descended asexually from identical micronuclei, can acquire different genetic properties during their development. He showed that the phenotype of *Paramecium* is determined by the macronucleus, not the micronucleus. He advanced our understanding of the states of immaturity, maturity, and senescence in the life cycle of the ciliated protozoa, showing that aging can be reversed

by autogamy as well as conjugation. His analysis of species in lower organisms produced novel evolutionary concepts.

Primarily he will be remembered for his studies on non-Mendelian inheritance. When he began his work, the role of the cytoplasm in heredity was entirely unknown. He showed that the various cases of non-Mendelian inheritance could be classified into distinct groups, most involving interactions between nuclear genes and the cytoplasm. His early studies on the cytoplasmic factor "kappa" established the first case of cytoplasmic inheritance in animals, and subsequent work by him and his students showed that intracellular symbiotes and cell organelles have become inextricably combined during evolution. His studies on surface proteins showed that complex systems of interacting elements in protein synthesis can create stable states of gene expression dependent on factors present in the cytoplasm. In a most elegant series of experiments on the ciliate cortex, he and his collaborators showed that the form and arrangement of preexisting structures determine the form and arrangement of new structures.

Finally, studying mating type and an unusual trichocyst mutant, he uncovered the first examples of a strange non-Mendelian phenomenon in which the macronucleus of ciliated protozoa determines the cytoplasm, and the cytoplasm in turn determines newly forming macronuclei, thereby passing genetic information from the old disintegrating macronucleus to the newly forming macronuclei.

PERSONAL HISTORY

Tracy Morton Sonneborn was born on October 19, 1905, in Baltimore, Maryland. His mother was Daisy Bamberger, and his father, Lee, was a businessman. Both encouraged him in his education. Others having an important influ-

ence in his early life were his uncle, Jacob Bamberger, and his cousin, Louis Bamberger. It was Louis Bamberger who established the Institute for Advanced Study at Princeton. As a teenager, Tracy became interested in the humanities and religion and at one point seriously considered becoming a rabbi. However, his beliefs soon changed, and, after attending Baltimore Polytechnic High School for two years and Baltimore City College High School for two more years, he entered Johns Hopkins University with the intention of studying literature. His interests changed to science when he took an introductory course in biology taught by E. A. Andrews. He received the B.A. degree from Hopkins in 1925.

He then began graduate work on the flatworm, *Stenostomum*, under the supervision of Herbert S. Jennings, director of the Zoological Laboratories at Johns Hopkins. Jennings was a remarkable scholar, one of the pioneers of biology. He published extensively and was renowned as a scientist, philosopher, and educator. Jennings had a broad view of biology. He worked on lower organisms and was concerned with the most fundamental aspects of behavior, inheritance, development, population biology, and evolution. Jennings had a profound influence on Tracy's development as a scientist. Tracy's passion for thoroughness and detail and his broad view of biology were like that of his teacher. He received the Ph.D at Johns Hopkins in 1928.

At that time, he received a National Research Council fellowship and spent 1928 and 1929 with Jennings at Hopkins working on the ciliate, *Colpidium*. In 1929 he married Ruth Meyers; it was a happy union that lasted until his death fifty-two years later. At the end of Tracy's fellowship in 1930, his attempts to obtain a faculty position failed, but he was offered a position as a research assistant at Hopkins with Jennings, who had just obtained a research grant from the

Rockefeller Foundation for work on *Paramecium*. During the period 1930 to 1939, Tracy held the positions of research associate and then associate at Johns Hopkins. After seven years of basic studies on the life cycle of *Paramecium*, he made his discovery of mating types in 1937, which immediately won him fame as an investigator. In 1939 Fernandus Payne persuaded him to accept a position at Indiana University as an associate professor. There he stayed for the rest of his life, becoming professor in 1943, distinguished service professor in 1953, and distinguished professor emeritus in 1976.

His first son, Lee, was born in 1929 in Baltimore and became a mathematician. His second son, David, was born in 1934, also in Baltimore and, like his father, became a biologist. Tracy's family life was remarkable. His wife, Ruth, was educated as a social worker and might have had a distinguished career of her own. Instead, she devoted her life to family and to his career. He was deeply grateful to Ruth, for she made it possible for him to devote himself virtually full time to his scholarly activities. She was clearly the mother and personal confidant of all the many students and postdoctorals who passed through the Sonneborn Laboratory at Indiana University. When Tracy arrived home from work, his role of eminent scientist whose every word was carefully considered by his students changed completely. He was just one more member, albeit a greatly beloved member, of a very close, well-adjusted, happy family. At one point during Thanksgiving dinner at my first visit to his home, amid all the gay conversation, Tracy was finally able to get in an opinion on the topic at hand. There followed a sudden silence around the table followed by a pronouncement from his youngest son, age five: "Old dummy Daddy." As a new graduate student I was indeed shocked, for at the laboratory his every pronouncement was worthy of the utmost

respect and consideration, but here everyone thought it was a splendid joke. Throughout his life these close relations within his family never changed.

Tracy was vitally interested in the activities and accomplishments of those about him. In conversation he spoke quickly and thought even more quickly. His incisive and often blunt comments were a bit intimidating at first for some of his new students, but his kindness and humor made him easy to engage in conversation. After a full day in his office and laboratory, he spent almost every evening thinking, writing, and making notes. For most of his life he met for a long session once a week in the evening with his students and others in his research group. Music and birds were his primary hobbies, but they took only a small portion of his time.

Every task that claimed his attention—an experiment, a new course, a research report, a manuscript to review, a student's class paper—somehow became the most important thing in the world to him. It had to be done with thoroughness and perfection. Nothing was too much trouble. An undergraduate lecture was as important as a keynote address at a major scientific meeting. He regularly took his place at undergraduate registration, interviewing each student (often 200 or 300), making sure all had the appropriate background and interests for his class. He once commented that teaching and research in no way interfered with each other, for all one needs to do is devote forty hours per week to each. For him that was clearly an understatement.

His lectures, whether for large classes, small classes, undergraduates, graduates, or scientific papers presented to his peers, were presented in a clear and exciting fashion. His enormous enthusiasm spread to all his audience. After a lecture at Goucher College in 1937 describing his first

finding of mating types, his audience was ready to follow him out the door and back to his laboratory to find what the experiments in progress would show. His first course at Indiana University, which was supposed to cover all the invertebrates, got no farther than the flatworms. He used to joke that when his department chairman, Fernandus Payne, learned that he had only covered protozoa, coelenterates, and flatworms in his course on invertebrates he almost got fired. It is noteworthy that two members of the class went on to careers studying protozoa, one even shifting from a commitment in another field. The excitement he generated was genuine and long lasting. For example, when he lectured on algae in a course with no formal laboratory, it was routine to see algae appear spontaneously in the various laboratories in which the graduate students worked, as they attempted to repeat and carry some of the experiments a step further.

In the late 1940s his laboratory enlarged. He brought in Wilhelm van Wagtendonk, a biochemist from Oregon, who Sonneborn hoped would work out the biochemical basis for the many genetic traits that he was investigating. However, it turned out that these traits were not readily accessible to biochemical investigation. Van Wagtendonk decided that it was necessary first to develop a defined medium for culturing *Paramecium*. This endeavor proved to be very difficult and time consuming. In the end he was successful, but it required the remaining portion of Van Wagtendonk's research career to achieve success. Early on, Ruth Dippell became his research technician. Ruth eventually received the doctorate degree and became a faculty member, but she always worked closely with him in her research. As the laboratory enlarged and his Ph.D. students increased in number, numerous postdoctoral workers also came, many from Europe and some from Japan and China. Bloomington

became the Mecca for all who would work on *Paramecium*. These investigators went on to important positions in universities and research institutes throughout the world. Soon most of the work on *Paramecium* was being done by those who had passed through his laboratory.

He continued to do research until his death in Bloomington in 1981, following a short illness with cancer.

Tracy received many honors during his career. He was elected a member of the National Academy of Sciences in 1946, a foreign member of the Royal Society of London in 1964, a member of the American Academy of Arts and Sciences in 1946, and a member of the American Philosophical Society in 1952. He received the Kimber Genetics Award of the National Academy of Sciences in 1939, the Mendel Centennial Medal of the Czechoslovakian Academy of Sciences in 1965, and the Newcomb-Cleveland Medal and Prize of the American Association for the Advancement of Science in 1946. He was an honorary member of the French Society of Protozoology, the Genetics Society of Japan, and the American Society of Protozoologists. He received honorary doctor's degrees from Johns Hopkins University, Northwestern University, Indiana University, the University of Geneva (Switzerland), and the University of Westphalia (Germany). He served as president or board member of many scientific organizations and gave numerous prestigious lectures in this country and abroad.

PROFESSIONAL HISTORY

STENOSTOMUM

When Tracy began his work for the Ph.D. in 1926, his mentor, Jennings, believed that, although Mendelian genes were responsible for most of the traits in higher organisms, other genetic mechanisms might also exist. These factors

were thought to be especially important in lower organisms, and it was also thought that they might be localized in the cytoplasm and susceptible to environmental modification. The way to test these speculations was simply to study the effects of environment and heredity on the development of various traits in selected lower forms of life. Such studies were to form the basis of Tracy's whole research career. His Ph.D. problem was on inheritance in *Stenostomum*, which reproduces asexually by dividing transversely into an anterior and a posterior half. He was able to identify and follow these halves in isolation cultures and found that progressive lines of anterior division products were more likely to age and die than lines of posterior products. He also exposed *Stenostomum* to lead acetate and found that abnormalities appeared. After such treatments he was able to isolate two-headed "monsters" that reproduced true to type. Since these traits were maintained for many generations, they were judged to have a hereditary basis. However, these variants arose and were lost at a much higher frequency than one would expect if they were due to mutations in simple Mendelian genes.

COLPIDIUM

After his Ph.D. work, Tracy stayed for eleven more years in Jennings's laboratory at Johns Hopkins. His first work was on the small ciliate, *Colpidium*, an organism he had used to feed his *Stenostomum*. He cultured *Colpidium* on a strain of bacteria on which they flourished. When he changed the bacterium to another less favorable kind, abnormalities appeared in the body form. From these abnormal animals he was able to isolate double animals, and these doubles reproduced true to type indefinitely, even when they were returned to culture on the more favorable bacterium. Again, the effect of the environment in inducing abnormal ani-

mals of a particular kind in high frequency was not what one would expect on the basis of mutation in Mendelian genes.

THE LIFE CYCLE OF *PARAMECIUM*

Sonneborn began his work on *Paramecium* when the problems of genetics, development, cell biology, and evolution were being attacked energetically by such workers as Morgan, Sturtevant, Bridges, Darlington, Haldane, Wright, Demerec, Jennings, Ephrussi, Beadle, Tatum, Emerson, McClintock, and Stadler. Sonneborn assumed his position as one of that group. His plan for research was simple: learn all he could about a single organism and apply his knowledge generally where applicable. By choosing a single organism, *Paramecium*, he thought he could attain a mastery of that organism that would enable him to carry out sophisticated experiments impossible for scientists who pick a single problem and move from organism to organism. He stuck to his plan faithfully, studying *Paramecium* almost exclusively during his whole research career. Sonneborn noted that, while protozoa are whole organisms, they are also single cells, and he recognized a rare chance to study inheritance independently of the complex multicellular life cycle that precluded investigations of cellular genetics in most organisms. While procaryotes are also unicellular, he felt that most studies on bacteria were concerned with populations of cells, not individual cells.

A test for Mendelism by breeding analysis could not be made in the case of either *Stenostomum* or *Colpidium* because both lacked sexual reproduction. By turning to *Paramecium*, which is able to conjugate and exchange germinal nuclei, he thought definitive tests of Mendelism would be possible. The only problem was that mating reactions, while common in both nature and the laboratory, could not be

controlled and often occurred even in clones (i.e., cultures derived by binary fission from single cells). So Sonneborn set about learning to understand and control mating and the life cycle.

Members of the *Paramecium aurelia* complex of species have a vegetative polyploid macronucleus that directly controls the characters of the cell and also two germinal micronuclei that periodically give rise to new macronuclei. *Paramecium* reproduces vegetatively by binary fission. The macronucleus divides amitotically, and the micronuclei divide by mitosis. At conjugation and autogamy, the old macronucleus breaks into fragments and normally is lost during subsequent fissions, while the two micronuclei undergo meiosis. A single haploid meiotic nucleus then divides to give a migratory and a stationary haploid nucleus. The migratory nucleus from each conjugant fuses with the stationary nucleus of its mate, or in the uniparental process of autogamy the two products simply fuse with each other. In each cell the diploid zygote micronucleus gives rise by mitosis to four micronuclei. Two remain as micronuclei and two develop independently into macronuclei. At the next fission the two new macronuclei are segregated one to each daughter cell, while the micronuclei divide mitotically and are distributed two to each daughter cell, restoring the normal vegetative state. Sonneborn was able to control autogamy when he found that a rapid fission rate in an excess of fresh culture medium inhibited autogamy while starvation induced it, provided the animals had undergone a sufficient number of fissions since the last conjugation or autogamy. Note that following the first fission after autogamy and conjugation each of the two cells has a macronucleus derived independently from different micronuclei—he called the two lines "caryonides." He discovered that mating within a caryonide is seen only rarely, while for the strains of *Para-*

mecium that he was studying, mixing cells of different caryonides in the proper physiological condition often resulted in immediate and massive mating reactions leading to pair formation and conjugation. In this way he not only discovered mating types in protozoa, but acquired the ability to make crosses between different lines. Many different mating types, characteristic of different strains of *P. aurelia* were described. The discovery of mating types was an exciting discovery and won Sonneborn immediate recognition by the academic community and even in the press.

Later he showed that sometimes fragments of the old macronucleus are not lost but persist and in subsequent asexual generations regenerate into macronuclei. Moreover, he learned how to induce this process of macronuclear regeneration at will. Although cytoplasm is not normally exchanged at conjugation, he also learned how to induce cytoplasmic exchange. Furthermore, he proved that the nuclei behaved as described above by showing that, after autogamy, lines are homozygous in all their genes, and after conjugation typical Mendelian ratios could be produced. These techniques gave him exquisite control over his organism and made it possible for him to carry out highly sophisticated genetic experiments.

MATING-TYPE INHERITANCE

As he continued his investigations on the genetics of *Paramecium*, Sonneborn studied all the character differences he could find. Unlike students of genetics in organisms like *Drosophila*, maize, and, later, *Neurospora* and yeast, he found that virtually every character he looked at in those early days proved to involve a combination of Mendelian and non-Mendelian elements. In some strains of *Paramecium* two mating types were found. Determination occurred at the formation of the new macronuclei at conjugation or auto-

gamy. Other strains, expressed only one mating type. It was shown that the difference in strains was accounted for by a single genic difference, the first gene demonstrated in ciliates. Today we know that massive reorganization of the DNA occurs at macronuclear formation in the ciliates, involving chromosome breakage, deletions, and reordering of sequences. In the case of mating-type determination, reorganization can proceed in such a way that one mating type is expressed in one caryonide, while another mating type is expressed in a sister caryonide. Only today are we coming to appreciate the many cases of nuclear differentiation that occur during development in the metazoa.

In simple caryonidal inheritance, mating type is determined independently of the parental type and independently of each of the two sister caryonides after conjugation or autogamy. However, it was found that, in some strains of *Paramecium*, mating-type inheritance proved to be caryonidal but also showed a marked tendency for the new caryonides to be like each other and like the mating type of the original cell in which they were formed. The results appeared to indicate cytoplasmic inheritance, and this conclusion was reinforced by crosses involving cytoplasmic transfer from one mate to the other. In a brilliant experiment involving conjugation, cytoplasmic exchange, and macronuclear regeneration, Sonneborn was able to produce individual cells that contained fragments of the old macronucleus destined to regenerate, micronuclei that were developing into macronuclei, and cytoplasm of the opposite mating type derived from the mate. At subsequent fissions, macronuclei of the two kinds segregated, and by means of genetic markers he was able to distinguish those derived from the old macronuclear fragments from those arising from new macronuclei developing in the normal way from micronuclei. The results demonstrated that newly forming macro-

nuclei derived from micronuclei respond to the cytoplasm in which they are found and become determined like the cytoplasm that surrounds them. However, the type of fragments is always like that of the original macronucleus from which they were derived. In short, in the formation of new macronuclei the cytoplasm determines the macronucleus for mating type, and once the macronucleus is determined it never changes. The cytoplasm, on the other hand, always reflects the type of macronucleus with which it is found. Thus, it appears that genetic information is passed from the old macronucleus to the cytoplasm to the newly forming macronuclei. This mode of inheritance has since been called "macronuclear inheritance" by Meyer. Macronuclear inheritance has been shown to occur for a number of other traits in *Paramecium*. Its molecular mechanism is still not understood.

KILLERS

Sonneborn also discovered and studied killers, paramecia that produced a toxin that could kill other strains of paramecia yet that are resistant to their own toxin. Crosses showed the presence of nuclear genes necessary for the perpetuation of the killer trait and also showed the presence of an essential cytoplasmic element that he called "kappa." Strains that lost kappa became sensitive to the toxin. Kappa proved puzzling to Sonneborn for many years, but it was finally shown in other laboratories that kappa is an example of a symbiotic bacterium able to live only in *Paramecium*. Many such forms have been described with various degrees of benefit and harm to their hosts. They emphasize to all geneticists the difficulties of distinguishing between infection and cytoplasmic heredity. In fact, it is now considered that all cases of cytoplasmic heredity based on the presence of self-replicating cytoplasmic nucleic acids are

probably derived evolutionarily from viruses or bacteria. Even such "normal" organelles as mitochondria and chloroplasts are thought to have such an origin.

SEROTYPES

Early workers showed that antiserum prepared by injecting paramecia into rabbits initially mobilized all cells of the injected clone. It was also found that resistant cells often appeared and that after isolation they produced resistant clones. Sonneborn began a study of this phenomenon and quickly confirmed the general features of the early studies. By injecting the resistant paramecia into rabbits, he obtained new sera and eventually showed that from a single clone of *Paramecium* he could obtain many subclones pure for up to a dozen different antigenic types, called serotypes, each reacting only with its own homologous antiserum. Moreover, he showed that exposure to antiserum actually induced the shift from one serotype to another. Genetic analysis revealed a series of independent genetic loci, each specific for a given serotype, with one gene active at a time. The shift from one serotype to another was due to switching from the activity of one gene (all the others inactive) to the activity of another. Serotype specificity and the ability of a serotype to be expressed at all were shown to be due to alleles at the different serotype loci. In one set of environmental conditions, Sonneborn found that most of the serotypes would reproduce stably for many generations. Crosses between serotypes of a single pure genotype always revealed cytoplasmic inheritance. Early in the investigation of serotypes it was pointed out that serotype inheritance could be explained in terms of stable states of gene expression that rely on feedback mechanisms for their perpetuation. This

interpretation was eventually accepted by Sonneborn and has recently received support from molecular studies.

PLASMAGENESE

Virtually *all* the traits Sonneborn encountered in his early studies were non-Mendelian, with strong genic and strong cytoplasmic components. At this point the evidence seemed to lead to the conclusion that cytoplasmic inheritance was an important component in all cases of inheritance in *Paramecium*. Perhaps in higher organisms that same was true, but it was being masked by the processes occurring in embryological development. So at this time the plasmagene theory was born: It was postulated that all genes in all organisms produce a self-reproducing entity that persists through somatic cell divisions but that is lost during sexual reproduction. The theory was given support by a number of studies done by others, especially the studies of Spiegelman on adaptive enzymes in yeast.

As work progressed, however, it became clear that the interpretation of the data as evidence for plasmagenes was not valid. Kappa and its relatives turned out to be symbiotic bacteria, dependent upon special genes for their maintenance. Further work on the expression of genes for surface proteins seemed to be best interpreted as a special interplay of competing inhibitors and activators of protein synthesis. Mating-type inheritance was more difficult to evaluate, but Sonneborn was able to show that mating-type inheritance was ultimately under nuclear control, the cytoplasm acting only to transmit information from the old macronucleus to the newly developing macronucleus. There was, in fact, no evidence for self-reproducing cytoplasmic genes.

THE CORTEX

Faced with these new findings, the notion of plasmagenes was discarded, and Sonneborn embarked on his investigations of the structure of the cellular cortex in *Paramecium*. Sonneborn always viewed his early work on *Stenostomum* and *Colpidium* as incomplete, for, although his two-headed monsters in *Stenostomum* and doublets in *Colpidium* arose in high frequency and in response to environmental stimuli in a decidedly non-Mendelian fashion, the organisms were asexual, and decisive breeding tests were not possible. He found, however, that doublets could easily be induced in *Paramecium* by exposing conjugating cells to antiserum. He now set about crossing singles with doubles. The results ruled out Mendelian genes. He also ruled out both the presence of determinants in the fluid cytoplasm and macronuclear inheritance like that observed for certain mating types. He was left with the cortical structure itself as the basis for the inheritance. Moreover, he and his collaborators were able to show that rearrangements in the pattern of the cilia, trichocysts, parosomal sacs, and fibrillar structures that make up the cortex also can be inherited in the same fashion. Sonneborn said that these instances were based on a new principle of inheritance that he called "cytotaxis," the ability of preexisting structures to control the formation and placement of new structures. Cytotaxis has since been studied extensively in the ciliate cortex by many workers.

Again, Sonneborn produced a brilliant series of experiments. They showed without doubt that preexisting structure controls the way new structures are formed in the cortex of ciliated protozoans. This work was held to be a major new phenomenon in genetics and development, applicable to all organisms. Currently, it appears that these principles

are indeed applicable to other organisms and organelles, but its true general significance is yet to be determined.

SENESCENCE AND THE LIFE CYCLE

It has been known for many years that after conjugation many ciliates undergo an immature period of many vegetative generations in which they are unable to mate. Then after a period of maturity, if mating does not occur, there is a period of senescence and finally death. Jennings pointed out that each stage lasts for such a long period that one must consider the stages heritable. The basis for the changes has remained unknown, although recent experimental evidence involving microinjection reinforces the view that its basis lies within the macronucleus. In any case, it is clear that the mechanism does not rely on simple Mendelian genetics. Sonneborn investigated the matter in relation to the unisexual process of autogamy in *Paramecium*. He found that autogamy could substitute for conjugation in rejuvenating senescent lines of paramecia. Another life-cycle change he noted was that after autogamy paramecia must undergo a certain number of fissions, in some cases a large number, before cells can undergo another autogamy. The basis for these life-cycle changes is unknown.

THE SPECIES PROBLEM

When Sonneborn discovered mating types, he found twenty-eight types among different strains. He was able to show that only mating type I could mate with mating type II, only III with IV, and so on, for a total of fourteen different mating pairs. He noted that each pair constituted a single interbreeding group. Since each group shared a common gene pool, it was clear that they constituted a series of sibling species. From the beginning he realized the taxonomic problem presented by the situation, for mating types can-

not be readily ascertained in the field. Even in the laboratory the process is time consuming, requiring the isolation and mixing of clones with standard mating types. He realized the problem that would be presented to taxonomists if the groups were given binomial names. His initial solution was to call the groups varieties. Later, in recognition of the genetic isolation of the varieties, he changed the designation to a newly invented term, "syngen." Finally, as more became known about the syngens, particularly their isozymes, responses to different strains of killers, fission rate, and other traits, Sonneborn recognized the syngens of *Paramecium aurelia* as separate species and designated them *P. primaurelia*, *P. biaurelia*, and so on. In other less-well-characterized ciliates, the mating groups are still called syngens.

Sonneborn also pointed out that the sibling species of the *P. aurelia* group, as well as the sibling species of other protozoans that are delimited primarily by their mating types, presented a set of interesting ecological and evolutionary problems. He noted that some species in the *P. aurelia* group have a long immature period after conjugation, while others have a very short or no immature period. Since those with a long immature period are less likely to mate with each other in nature, he classified them as "outbreeders," while those with a short immature period he classified as "inbreeders." Outbreeding, which favors genetic diversity, was held to be the ancestral type. He made detailed studies of the properties of the various species and also studied the viability of progeny obtained from crosses. He related this information to the ecology and evolution of the groups.

GENES

Different Mendelian genes were not readily found in *Paramecium*, but the behavior of the first ones that Sonneborn found were sufficient to establish the validity of the com-

plex cytological events of the life cycle. Eventually, it was found that numerous mutants could be isolated after chemical mutagenesis. Sonneborn then engaged in a large study, isolating more than 100 different visible morphological and behavioral mutants. These were then tested for linkage. Because of the large number of chromosomes in *Paramecium* and perhaps because of a high rate of recombination, few cases of linkage and no maps resulted.

TRICHOCYSTS

Sonneborn's last project was the investigation of an aberrant mutation that reduced the ability of trichocysts to discharge. Although several simple gene mutations had the same effect, this mutant seemed to follow the cytoplasm in crosses. A more detailed analysis revealed that it was inherited just as mating type was inherited in many strains—that is, it was macronuclear inheritance as described above. Since Sonneborn's death, additional cases of macronuclear inheritance affecting other traits have been found and are now being actively investigated.

CONCLUSION

While Sonneborn was learning whatever *Paramecium* could teach him about biology, a new generation of microbial geneticists, working with fungi, bacteria, and bacteriophages, was establishing the foundations for the new science of molecular biology. Unlike *Paramecium*, these organisms had properties that proved to be invaluable in the new science. They had simple nutritional requirements, synthesizing most of the complex substances they needed and thereby enabling the investigator to study the genetic control of many of the enzymes of metabolism. They could be plated onto agar, making possible the quick and easy examination of innumerable clones. This technique was absolutely es-

sential for the study of mutations or rare recombinants. In these organisms one could carry out studies on the role of genes in controlling metabolic pathways, enzyme synthesis, and enzyme structure. Investigations of mutations and genetic fine structure also were possible. These were the studies that finally led to our knowledge of the roles of DNA and RNA and produced the modern revolution in molecular biology. *Paramecium* was eminently unsuited for any of these studies.

Hence, Sonneborn did not participate in this revolution that was sweeping biology and biochemistry, although it was clear that, like everyone else, he greatly appreciated and admired the work that was going on. He would have loved to be at its forefront. But *Paramecium* did not lead him there and could not have led him there, for it was simply not useful for such studies. The Nobel prizes that were awarded so generously to the disciples of the new biology eluded Sonneborn. That is not to say that his work was unnoticed. He was, indeed, widely recognized as an outstanding investigator. Nevertheless, a glance at any current textbook of general biology or genetics leads one to the conclusion that he was not the originator of concepts that are basic to the thinking of most biologists and geneticists today.

It has been suggested that Sonneborn avoided more conventional genetics and focused on the role of the cytoplasm in heredity. In my view, that notion is not correct. Sonneborn concentrated on the inheritance of whatever traits he could find in *Paramecium* without prejudice. It simply turns out that most of the easily observable traits in *Paramecium* are inherited in a non-Mendelian fashion. Would he have pursued his research differently had he known that *Paramecium* could not take him to the forefront of the great revolution in biology that was just developing? In those early days no

one knew what *Paramecium* had to offer. It was a member of a group of organisms that was simply too big and too different to be left unexplored. We had to know what protozoa were like, just as we had to know about bacteria and viruses and insects and mice and corn and worms and zebra fish. We had to know about the role of the cytoplasm in genetics. And, indeed, *Paramecium* turned out to be ideal for the study of inheritance at the cellular level and for the study of nuclear differentiation. Although there are no plasmagenes, there are cytoplasmic entities that contain DNA. There are stable metabolic states that are passed from one generation of cells to the next. Preexisting structures and patterns of structures are important in determining new structures at cell division. And, finally, differentiation of new nuclei in ciliates can produce new stable configurations and can be influenced by factors emanating from preexisting nuclei and passed through the cytoplasm. The role of preexisting structure in developmental biology is not yet understood, and the strange nuclear and cytoplasmic effects that Sonneborn uncovered are still unexplained at the molecular level. Whatever the final outcome of studies of these phenomena, he must take his place among the most brilliant and devoted experimentalists in the history of biology and a true giant, like no other, in the field of protozoan research.

I HAVE DRAWN ON unpublished material in my files, much received from Tracy himself over the years, as well as unpublished material from Ruth Dippell and Ruth Sonneborn, his wife. The reader is also referred to an account of Tracy's life by G. H. Beale in *Biographical Memoirs of Fellows of the Royal Society*, vol. 28, pp. 537-74 (London: Royal Society, 1982).

SELECTED BIBLIOGRAPHY

1930

Genetic studies on *Stenostomum incaudatum* (nov. spec.), II. The effects of lead acetate on the hereditary constitution. *J. Exp. Zool.* 57:409-39.

1932

Experimental production of chains and its genetic consequences in the ciliate protozoan *Colpidium campylum*. *Biol. Bull.* 63:187-211.

1937

Sex, sex inheritance and sex determination in *Paramecium aurelia*. *Proc. Natl. Acad. Sci. U.S.A.* 23:378-85.

1939

Paramecium aurelia: mating types and groups; lethal interactions; determination and inheritance. *Am. Nat.* 73:390-412.

1941

Relation of macronuclear regeneration in *Paramecium aurelia* to macronuclear structure, amitosis and genetic determination. *The Collecting Net* 16:3-4.

Sexuality in unicellular organisms. In *Protozoa in Biological Research*, ed. G. N. Calkins and F. M. Summers, pp. 666-709. New York: Columbia University Press.

1943

Gene and cytoplasm. I. The determination and inheritance of the killer character in variety 4 of *P. aurelia*. *Proc. Natl. Acad. Sci. U.S.A.* 29:329-38.

Gene and cytoplasm. II. The bearing of the determination and inheritance of characters in *P. aurelia* on the problems of cytoplasmic inheritance, *Pneumococcus* transformations, mutations and development. *Proc. Natl. Acad. Sci. U.S.A.* 29:338-43.

1945

Gene action in *Paramecium*. *Ann. Mo. Bot. Garden* 32:213-21.

1946

Experimental control of the concentration of cytoplasmic genetic factors in *Paramecium*. *Cold Spring Harbor Symp. Quant. Biol.* 11:236-55.

1947

Recent advances in the genetics of *Paramecium* and *Euplotes*. *Adv. Genet.* 1:263-358.

1948

The determination of hereditary antigenic differences in genically identical *Paramecium* cells. *Proc. Natl. Acad. Sci. U.S.A.* 34:413-18.

With A. LeSeur. Antigenic characters in *Paramecium aurelia* (variety 4): determination, inheritance and induced mutations. *Am. Nat.* 82:69-78.

1950

Methods in the general biology and genetics of *Paramecium aurelia*. *J. Exp. Zool.* 113:87-148.

Beyond the gene—two years later. In *Science in Progress*, ed. G. A. Baitsell, pp. 167-203. New Haven: Yale University Press.

1954

The relation of autogamy to senescence and rejuvenescence in *P. aurelia*. *J. Protozool.* 1:36-53.

1957

Breeding systems, reproductive methods, and species problems in protozoa. In *The Species Problem*, ed. E. Mayr, pp. 155-324. Washington, D.C.: American Association for the Advancement of Science.

1959

Kappa and related particles in *Paramecium*. *Adv. Virus Res.* 6:229-356.

1962

Does preformed cell structure play an essential role in cell hered-

ity? In *The Nature of Biological Diversity*, ed. J. M. Allen, pp. 165-221. New York: McGraw-Hill.

1965

With J. Beisson. Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. *Proc. Natl. Acad. Sci. U.S.A.* 53:275-82.

1970

Methods in *Paramecium* research. In *Methods in Cell Physiology*, vol. 4, ed. D. Prescott, pp. 241-339. New York: Academic Press.

1974

Paramecium aurelia. In *Handbook of Genetics*, vol. II, ed. R. King, pp. 469-594. New York: Plenum Press.

1975

The *Paramecium aurelia* complex of fourteen sibling species. *Trans. Am. Micros. Soc.* 94:155-78.

1977

Local differentiations of the cell surface of ciliates: their determination, effects and genetics. In *The Synthesis, Assembly and Turnover of Cell Surface Components*, ed. G. Poste and G. L. Nicholson, *Cell Surface Reviews*, vol. 4, pp. 829-56. New York: Elsevier/North Holland.

1979

With M. V. Schneller. A genetic system for alternative stable characteristics in genomically identical homozygous clones. *Dev. Genet.* 1:21-46.

1980

With Y. Brygoo, A. M. Keller, R. V. Dippell, and M. V. Schneller. Genetic analysis of mating type differentiation in *Paramecium tetraurelia*. II. Role of the micronuclei in mating-type determination. *Genetics* 94:951-59.