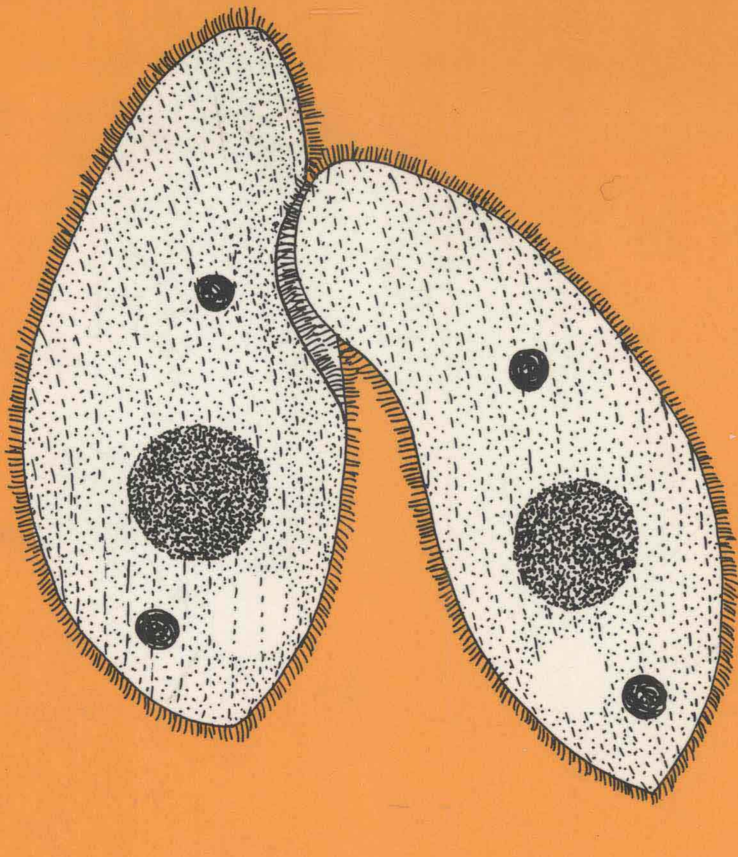
SEX and DEATH in PROTOZOA

The History of an Obsession



Graham Bell

SEX AND DEATH IN PROTOZOA

The history of an obsession

GRAHAM BELL

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Sex and death in protozoa

For my mother

PREFACE

This book began when, out of curiosity, I glanced at the contents of the first issue of the Quarterly Review of Biology and saw an article with the irresistible title of 'Eleven thousand generations of *Paramecium*'. Its author, L. L. Woodruff, was describing how he had maintained a strain of *Paramecium aurelia* in isolate culture for 19 years, with no sign of any decrease in vitality. My first thought was that this would provide an excellent illustration of the futility of research unguided by adequate theoretical understanding. On second thoughts, there might be more to the problem than met the eye; and little by little I was drawn into reading most of a huge literature, the bulk of it written between 1890 and 1920, generated by the speculation that sex has some ill-defined rejuvenating property that made it indispensable to the indefinite prolongation of a line of protozoans. Although most geneticists and protozoologists (I am neither) are aware of this literature, it has long since faded from the foreground, and the musty volumes in which it lies entombed are nowadays little read. I suspect that most people have been put off by the almost mystical properties ascribed to sex by many of these early workers, at a time when Mendelism had only just been rediscovered and the concept of the genotype was still novel. The attempts to 'revivify' failing cultures with extract of pancreas, for example, read rather strangely today. A large part of this book is simply historical, therefore, born of my fascination with the attempts of these early geneticists to grapple with ideas of sexuality and immortality, and of my own attempts to understand their results in modern terms. But this in turn led me to think more deeply about the limits to the fidelity with which genetic information can be transmitted through a very long sequence of generations, and this more general question forms the second facet of the book. I have tried to report my findings so that they can be understood by any moderately wellinformed reader, from the undergraduate level upwards; to the specialists who will be irritated by the necessary shallowness of many sections I offer my apologies in advance.

This project was conceived, and most of the data retrieval and analysis completed, at McGill, where the Biology Department continues to offer an enormously stimulating intellectual milieu. It was supported in part by funds from the Natural Sciences and Engineering Research Council of Canada. Most of the text and a good part of the theory was done at the University of Sussex, who provided unstinted hospitality during my sabbatical leave. In particular, I have benefited more than I can readily express from conversations with John Maynard Smith. I only hope that he will forgive me for any ideas of his that he finds embedded without acknowledgment in the text; they will be the best ones.

Graham Bell
Montreal
November 1987

CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

1

The question of protozoan immortality

Birth and death are notoriously the only two certainties in life. Men have always desired immortality, have been willing to pay any price in order to procure it, and have always been denied it. They have not ceased to ask questions about it. Since the origins of scientific biology, two recurrent themes have been whether all organisms are born, and whether all must die; and the notion that there is a deep connection between these two invariants has fuelled speculation from the earliest times down to the present day. Spontaneous generation was gradually excised from the scientific curriculum, or at any rate pushed far back into the geological fog, but the possibility of immortality has not been so easy to dispose of. All our familiar animals and plants must surely die, as we must ourselves; even when protected against all the usual rigours of life, the approach of death is eventually signalled by a progressive deterioration, an irreversible process of senescence. It was not obvious that the same should be true of the new world of minute creatures revealed by the invention of the microscope towards the end of the seventeenth century. Protozoans like the *Amoeba* and *Paramecium* familiar from introductory biology classes grow, divide into two apparently identical cells, grow and divide again. They may readily be killed, of course, by almost any sort of minor physical or chemical insult, but in principle, given favourable conditions and protected from shocks, perhaps the cycle of growth and division would continue indefinitely, and thereby prove that natural death was not after all inevitable. Ehrenberg (1838) appears to have been the first to publish this speculation, and by doing so ushered in one of the great debates of biology.

Ehrenberg's suggestion was opposed by Engelmann (1862, 1876) and Butschli (1876), who found that after a long time in culture ciliate protozoans begin to show morphological abnormalities which they interpreted as senescent degeneration akin to that of metazoan cells. At the same time, it was shown that during sexual conjugation in these hypotrichs – first correctly interpreted by these authors – many organelles were resorbed and later regenerated. They proposed, therefore, that protozoans were mortal just as metazoans were, but that, like the metazoan egg, they were rejuvenated by sex. Engelmann put this very strongly: 'The demonstration of this process of reorganization, of rejuvenescence, makes it, as it seems to me, entirely unnecessary to search for any other effect of conjugation' (Engelmann, 1876). This 'rejuvenescence', in the subsequent development of the theory, might occur in either of two ways: by a chemical stimulation caused by the mixing of two dissimilar protoplasts, in a manner analogous to the activation of eggs by artificial parthenogenesis, a subject brought into great vogue at about this time by Jacques Loeb; or by a change in the 'fundamental organization' of the products of conjugation, just as embryogenesis normally requires contributions from two parents.

These views were memorably opposed by August Weismann (1889, 1891; see Kirkwood and Cremer, 1982), on the basis of his ideas about the independence of the germ plasm from the soma. Weismann argued that any organism is bound to suffer a series of minor accidents whose cumulative effect will at last be seriously weakening. Senescence evolves as a device to rid the species of worn out, reproductively valueless individuals and allow them to be replaced by their younger and more vigorous progeny. Since the immortality of somatic cells would be useless or even damaging, selection would not hinder the evolution of specialized cell types, which being unable to replicate themselves, were bound to die. Indeed, senescence would be of immediate advantage because materials and energy diverted from the increasingly expensive task of maintaining aging somatic cells could be more profitably deployed to increase reproductive output. In these ideas we can see the germs of several modern theories, including the 'mutation–accumulation' theory of aging suggested by Edney and Gill (1968), and Williams' concept of life history evolution (Williams, 1957). Germ cells, however, must be an exception, since if they were to age the species would become extinct; they therefore retain a vicarious immortality by virtue of their passage from generation to generation. Now, protozoans – he argued – are all potential germ cells, capable of giving rise to new progeny by fission, and therefore share the immortality of the metazoan germ line. Sex, in Weismann's view, was a quite unrelated phenomenon, having no direct connection with senescence. Its function was rather to provide the variation on which continued evolution by natural selection depends.

However, despite Weismann's attempts to uncouple the problems of sex and death, by the last quarter of the nineteenth century they had become inextricably entangled in a single question, to which an answer would be strenuously sought for the next fifty years: could a line of protozoans be propagated indefinitely by vegetative fission, or was eventual extinction certain in the absence of sex? Some early investigations by Dujardin (1841) and Balbiani (1882) seemed to show a decline of vitality – the rate of vegetative fission – in aging cultures, but the generality of senescent decline and its reversal by sexual conjugation was not firmly established until the classical work by Maupas (1888, 1889). In his very extensive experiments he not only laid the technical and conceptual foundations for almost all subsequent work on the subject, but claimed to demonstrate that protozoan cultures pass through a life-cycle comparable to that of metazoans, except, of course, that it is the life-cycle of a clone rather than of an individual. Cultures may be maintained for several hundred generations, but eventually morphological degeneration, often a decrease in size, begins to set in. The animals begin to look shrunken and distorted; the rate of feeding drops; some structures may disappear entirely; even the micronucleus and macronucleus may dwindle and be lost. At last, worn out and enfeebled, the culture dies out. Just like an individual metazoan, the lineage descending from a single protozoan passes through the stages of youth, maturity, old age and inevitable death. These stages are most characteristically distinguished by the ability to undergo sexual conjugation, which is low in youth, greatest in maturity, and disappears in old age; Maupas himself neither consistently observed nor attributed much importance to the senescent decline in the rate of fission that was to be described by so many later authors. During maturity, conjugation arrested the process of senescence, restored youthful vigour to the cell, and thus made continued existence possible. Maupas' experiments, therefore, seemed to establish for sex the fundamental biological role of erasing in each generation the stains and creases of old age.

At about the same time, an independent series of observations was being made by Richard Hertwig (Hertwig 1903), who attempted to subsume his own and Maupas' results under a single general theory. As the life-cycle progresses, the volume of the nucleus increases relative to that of the cytoplasm, supposedly because the nucleus continually extracts and sequesters material from the cytoplasm. This leads eventually to a physiological imbalance which impairs function and induces senescence. Senescence is prevented, therefore, by any process which by reducing the volume of the nucleus restores an optimal 'nucleoplasmic ratio'. This can be achieved, for instance, by the fragmentation of the ciliate macronucleus and the resorption of some of these fragments by the cytoplasm. Such processes rarely restore the nucleus precisely to its proper size, however; it usually remains too large, and the optimal nucleoplasmic ratio must be recreated by further growth of the cytoplasm. (There is some discrepancy here between Maupas and Hertwig: Maupas' animals senesced by getting smaller, while Hertwig usually observed an increase of size in aging cultures.) Only sexual union was completely effective in restoring vigour, perhaps because the introduction of foreign material into the cell somehow slowed down the reaction between nucleus and cytoplasm. Hertwig did not claim that fertilization increased vegetative vigour; on the contrary, its immediate consequence would be to reduce the rate of fission until the foreign nuclear material became adjusted to the new cytoplasm in which it found itself.

Hertwig elaborated these ideas into a general theory of the life-cycle. For example, male gametes have a nucleus which is very large relative to the cytoplasm, while the reverse is true for female gametes; sexual fusion is therefore necessary to restore the optimal nucleoplasmic ratio. He was an influential figure, whose followers (e.g. Gerassimoff 1901, 1902; Popoff 1908, 1909; Rautmann 1909) gave rise to a large literature. During the 1920s, however, publications became fewer, and eventually work on the nucleoplasmic ratio ceased. It was instead Maupas' influence which continued to be felt, with the mainstream of research being devoted to long-term cultural studies.

Ironically, the great majority of these studies concerned themselves with a proposition repeatedly and explicitly denied by both Maupas and Hertwig–Butschli's contention that the leading characteristic of senescence in protozoan cultures was a gradual decline in the rate of fission, which could be reversed by sexual conjugation. Fabre-Domergue (1889) pointed out early in the debate that both a decline in the fission rate and Maupas' senile morphological changes could be caused by a slow deterioration in culture conditions. Joukowsky (1898) agreed, and managed to maintain *Paramecium* through some 450 generations, though his cultures eventually declined and died. The most vocal opponent of the life cycle concept was Enriques (1903, 1905), who took *Glaucoma* through nearly 700 generations without conjugation and with no decline in the fission rate. He attributed his success to frequent changes of medium; cultures kept in medium which was often allowed to grow stale declined and died in the usual Maupasian manner. Impressed by the tedium of transferring isolate lines to fresh medium every day, Enriques concluded that what becomes exhausted in most experiments is not the vitality of the organisms but the patience of the investigator.