Series

What history tells us
IV. Ciliates as models... of what?

MICHEL MORANGE

Centre Cavaillès, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, 33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

The major role of *Drosophila* in the rise of genetics, or of bacteria and bacteriophages in that of molecular biology, is familiar to all readers. Amphibians were decisive tools in the development of experimental embryology. More recently, the study of the nematode *Caenorhabditis elegans* and of the zebrafish has been closely associated with the new molecular developmental biology. All these organisms have served as models for biologists.

In most cases, an organism is a model for a limited period of time. It becomes a model either because it is the best 'object' to use in addressing a specific new set of questions, or because it is nicely adapted to the use of a new technology. In contrast to the previous examples, ciliates – and in particular paramecia – have remained model organisms for more than a century. They were not considered as models for the same reasons throughout this period, and it is possible to distinguish at least three successive and different uses of these organisms as models – at the end of the 19th century, in the middle of the 20th with the work of Tracy Sonneborn, and more recently in the molecular and post-molecular era. The second episode is familiar to historians of 20th century biology (Sapp 1987). Recent studies have enriched it, as well as thrown light on the first use of ciliates as models.

2. Unicellular organisms as objects of psychophysiological research

The interest in ciliates in the study of psychophysiology finds its origin in the development of cell theory in the middle of the 19th century, and the monist interpretation of evolutionary theory given by Ernst Haeckel: the first signs of psychological characteristics have to be looked for at the cellular level. Unicellular organisms as ciliates are the best organisms for such a project.

In their very original historical work, Judy Johns Schloegel and Henning Schmidgen (Schloegel and Schmidgen 2002) describe three successive research projects. The first, initiated by Max Verworn in Berlin and developed later in Jena in the mid 1880s, considered the spontaneous and induced movements of protists. Although Max Verworn interpreted most of his observations as the result of "impulsive and automatic movements", his microdissections and the spontaneous behaviour of the different parts of the cell pushed him to conclude that protozoa possessed elementary sensations and representations located in different parts of the protoplasm.

At the same time, in 1887, Alfred Binet – better known for his later invention of the intelligence scale – published a study on the "psychology of proto-organisms". Binet was much more enthusiastic than Max Verworn. He found in these microorganisms manifestations of an intelligence which greatly transcends the phenomenon of cellular irritability. His arguments were clearly anti-reductionist. When the famous physiologist Charles Richet objected that protozoa were not good models of cells because they were neither simple nor homogeneous, Binet answered that nothing such as a simple homogeneous cell exists in nature.

The American zoologist Herbert Spencer Jennings – who was the mentor of Tracy Sonneborn – went to Max Verworn's lab in Jena for his post-doctoral studies. Nevertheless, he did not adopt the vivisection technique of Verworn. In a series of ten papers published between 1897 and 1902, and in his book *Behavior of the lower organisms* (Jennings 1906), he interpreted the behaviour of paramecia – in particular the avoiding answer – as the result of a "trial and error" strategy, and he hypothesized the existence of a low level of consciousness in these organisms.

A fascinating part of the story, which is beyond the scope of the present article, is the impact that these studies had on

philosophers – Friedrich Nietzsche, Charles Peirce, Henri Bergson – and on Sigmund Freud through his encounter with Jennings.

3. Paramecia as the best model to go "beyond the gene"

The importance of studies on paramecia in the search for extragenic and/or extranuclear forms of heredity has already been well documented (Sapp 1987). Paramecia and ciliates are very complex cells, with well-organized cytoplasmic territories having different functions. The maintenance of these differentiated subcellular domains during cell division appeared to be a phenomenon similar to the maintenance of differentiated cells and structures in a multi-cellular organism; and similar mechanisms were looked for to explain both processes. Early studies by André Lwoff in the 1920s pressed him to conclude that kinetosomes – the source of cilia – are endowed with genetic continuity, and that the cortical structure commands morphogenesis. Forty years later, Janine Beisson and Tracy Sonneborn provided strong experimental arguments in favour of a cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia* (Beisson and Sonneborn 1965). In Germany, Victor Jollos had shown that the action of the environment could produce modifications persisting over hundreds of generations which he called dauermodifikationen, but these disappeared during conjugation. The major step which transformed ciliates into a model to go beyond the gene (Sonneborn 1949) was the discovery by Tracy Sonneborn in 1937 of the sexual types, and the precise description of the mechanisms of conjugation (Sonneborn 1937). The reciprocal exchange of meiotic nuclei generates two genetically identical organisms with different cytoplasms: an ideal system to observe the effects of a cytoplasmic form of inheritance on an identical genetic background. The partial exchange of cytoplasm that occurs in certain cases during conjugation provides another way to estimate the weight of genetic (nuclear) and extragenetic forms of heredity. In addition, ciliates have two different forms of nuclei, of very different sizes: the micronuclei are responsible for reproduction, whereas the macronuclei, which are derived from the micronuclei, are responsible for the visible phenotype. The two different functions of the genetic material in ciliates are therefore separated into two different substructures. It is only recently that the differences between the states of the genetic material in the micronucleus and macronucleus have begun to be described.

Soon after Sonneborn's description of the conjugation process, many hereditary phenomena were shown not to obey simple Mendelian rules, and to be in one way or another controlled by the cytoplasm: the antigenic types of paramecia, the capacity of certain strains to kill others owing to the existence of a kappa factor in their cytoplasm, and even the possession of one or other of the two mating types.

The first two observations led Sonneborn to support the plasmagene theory, according to which some hereditary phenomena are due to self-replicating particles present in the cytoplasm, themselves controlled by the nuclear genes. Because of this support, Sonneborn has been considered by many historians as heterodox, opposing the nuclear monopoly, taking the side of the cytoplasm in the long debate on the relative roles of the nucleus and cytoplasm in heredity. The plasmagene theory was a way to explain the stable cytoplasmic organization of ciliates as well as the stable state of gene expression in differentiated cells of a multicellular organism.

We will come back to the attitude of Sonneborn in the conclusion. As far as paramecia were concerned, two opposing processes occurred. Quite rapidly, the heterogeneity of the different observations became obvious. Kappa was shown to be a symbiotic bacterium. The stability of surface antigens resulted from the maintenance of an active or inactive state of gene expression of the different surface antigen – coding genes, whereas the choice of mating type is the result of the complex dialogue between the preexisting maternal macronucleus and the new macronucleus copied from the zygotic genome (Orias 1981). Additional 'bizarre', non-Mendelian phenomena were progressively discovered (see later).

4. Paramecia as a model for molecular biologists

From the complexity of the previous observations, paramecia were progressively abandoned as a model for genetic studies. The diminishing importance accorded to these organisms in genetic textbooks is obvious (Preer 1997). But it has not been always noticed that, from the molecular point of view, paramecia – and *Tetrahymena*, another ciliate, which can be easily cultivated on well-defined media – have frequently been the source of important novelties: the catalytic activity of RNAs was discovered by Thomas Cech when studying the splicing process of the ribosomal RNA precursor of *Tetrahymena* (Cech *et al* 1981; Kruger *et al* 1982). The first exception to the universality of the genetic code – apart from the case of mitochondria – was discovered in paramecia (Caron and Meyer 1985; Preer *et al* 1985). The characterization of telomerase, the enzyme which opposes the permanent reduction in length of the extremities of chromosomes, the telomeres, at each generation, was done in *Tetrahymena* (Greider and Blackburn 1985). This was not the fruit of chance: a huge number of telomeres is synthesized during the formation of the macronucleus from the micronucleus.

It is the latter phenomenon, the conversion of a micronucleus to a macronucleus, which has been the focus of

attention of the most recent molecular studies in paramecia. Not only are the micronuclear chromosomes fragmented to generate smaller macronuclear chromosomes, but a large number of internal sequences are precisely deleted during this process. These DNA rearrangements are reminiscent of what occurs during the maturation of the immune system in B and T cells. The influence of the maternal macronucleus on the zygotic macronucleus was clearly demonstrated in these studies, generating a whole new ensemble of phenomena pertaining to maternal heredity. Recently, the role of heterochromatin and small interfering RNAs was demonstrated, again making paramecia a model system for the study of epigenetic processes.

5. Conclusion

We have described the way(s) in which ciliates were considered as a biological model over a long time frame, of more than a century. Historians usually prefer to focus on a more limited period of time. In most cases of animal models, such a strategy is possible since the half-life of a model is rarely more than two or three decades. The ciliates are somehow exceptional. They are – and have always been – fascinating organisms: they have developed a specialization of intracellular structures and functions comparable to what has occurred between the different cells of a multicellular organism. The history of studies on ciliates and paramecia is full of furore and controversies, and rich too in wrong observations and false models (Hall 1998). It cannot be told independently of the particular people who made it: exceptional objects in science always attract exceptional characters (Nanney 1982), those who are not uniquely motivated by the art of the soluble, and not repelled by a flavour of heterodoxy. Ciliates are exceptional, but as Sonneborn used to say, quoting Bateson: "Treasure your exceptions" (Preer 1997).

What does this story tell us about the nature and the role of animal models in the biological sciences? There are probably two different kinds of models. We are more familiar with the first: those which have been characteristic of the 20th century (Davis 2003). For various technical reasons – small size, rate of reproduction, size of the genetic material – these models were the best suited to bring to light general rules operating in the living world: from the genetic code to the principles of development. In this sense, the paramecium model was not totally successful: this organism was rapidly abandoned as a model for the study of the basic principles of psychology – as was the fungus *Phycomyces* adopted by Max Delbrück when he turned away from the bacteriophage to look for the molecular bases of sensory physiology (Judson 1996). As a genetic model to outline the extranuclear contributions to heredity, paramecia also did not totally fulfil their promise. Extragenetic forms of heredity were described, but their complexity, their characteristics narrowly linked to the life-cycle of these organisms, made their study both difficult and of a limited general value.

But the attitude of biologists is rapidly changing (Davis 2003). First, for technological reasons: genome sequencing programmes have been extended to esoteric organisms – including paramecia – and this knowledge allows new experimental approaches to these organisms. Yet this renewed interest is probably more the result of a new way of looking at life and its diversity. The general rules of the game of life are now well known. What interests biologists is to see how the different organisms have exploited these rules to create new devices or find new solutions to functional problems. The organisms are now model organisms *per se* (Narasimhan 1999). And within this new meaning of a model, ciliates are wonderful objects. For a billion years they have been tinkering with their genetic and epigenetic mechanisms of heredity. The results are original, and differ from one ciliate to another. The study of these organisms shows that the clear boundary between genetic and epigenetic mechanisms of control – for instance during the formation of the macronucleus – is much fuzzier than initially thought: what is genetic in one species can be epigenetic in another. These organisms have not elaborated new mechanisms – histone modification and siRNAs are active players in the game – but they have diverted them to the solution of their specific problems (Nowacki *et al* 2005). It is this kind of richness and diversity which presently interests biologists, and which probably interested Sonneborn too. His catholic and organism-oriented approach was not understood by his contemporaries, who only retained from his studies the challenge they posed to the general models (Schloegel 1999).

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