
FRANCISCO AYALA AND THE PASSION FOR PARASITIC PROTISTS

Francisco Ayala y la pasión por los parásitos protistas

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ABSTRACT: Francisco J. Ayala, a renowned evolutionary biologist, made significant contributions to the study of parasitic protists, particularly in the fields of Chagas disease, leishmaniasis, and trypanosomiasis. His research revealed the multiclonal structure of *Trypanosoma cruzi* populations and the extreme DNA sequence differences among its isolates, which was crucial for understanding the prevention, spread, and natural foci of Chagas disease. Ayala and his collaborator Michel Tibayrenc formulated the Clonal Theory of Parasitic Protozoa, which challenged the prevailing view of sexual recombination in these organisms. Ayala's work on leishmaniasis described the predominantly clonal population structure of *Leishmania* parasites worldwide. Furthermore, his expertise in evolutionary biology helped resolve the taxonomic confusion surrounding *Trypanosoma equiperdum* and *Trypanosoma evansi*, supporting the hypothesis that they are forms of *Trypanosoma brucei*, the causative agent of sleeping sickness in Africa. Ayala's passion for studying parasitic protists stemmed from his desire to help alleviate human suffering caused by these diseases.

KEYWORDS: Parasitic Protists, *Trypanosoma cruzi*, Chagas disease, Leishmaniasis, evolutionary biology.

RESUMEN: Francisco J. Ayala, reputado biólogo evolutivo, realizó importantes contribuciones al estudio de los protistas parásitos, en particular en los campos de la enfermedad de Chagas, la leishmaniasis y la tripanosomiasis. Sus investigaciones revelaron la estructura multiclonal de las poblaciones de *Trypanosoma cruzi* y las diferencias extremas de secuencia de ADN entre sus aislados, lo que fue crucial para comprender la prevención, propagación y focos naturales de la enfermedad de Chagas. Ayala y su colaborador Michel Tibayrenc formularon la Teoría Clonal de los Protozoos Parásitos, que cuestionaba la visión predominante de la recombinación sexual en estos organismos. El trabajo de Ayala sobre la leishmaniasis describió la estructura de población predominantemente clonal de

los parásitos de *Leishmania* en todo el mundo. Además, su experiencia en biología evolutiva ayudó a resolver la confusión taxonómica en torno a *Trypanosoma equiperdum* y *Trypanosoma evansi*, apoyando la hipótesis de que son formas de *Trypanosoma brucei*, el agente causante de la enfermedad del sueño en África. La pasión de Ayala por el estudio de los protistas parásitos surgió de su deseo de contribuir a aliviar el sufrimiento humano causado por estas enfermedades.

PALABRAS CLAVE: Protistas parásitos, *Trypanosoma cruzi*, enfermedad de Chagas, leishmaniasis, biología evolutiva.

Eukaryotes are organisms with cells containing a nucleus and numerous other compartments. They include microscopic organisms, as well as all those visible by naked eye, including insects, sequoia trees and humans. Hence, it may come as a surprise that the bulk of eukaryotic diversity is hidden in so-called unicellular eukaryotes, i.e. protists (previously also termed protozoans). It is of course not morphological diversity, because few micrometers long cells can hardly carry as many distinguishing features as for example plants in a tropical forest, but it is the diversity of their molecular and cellular mechanisms. Indeed, two protistan species belonging to one genus, such as f.e. *Trypanosoma*, may differ from each other in their DNA sequences as much as an elephant differ from a cat. It is the constraint of their size that these stunning differences on the molecular level do not reflect on their morphology. The diversity of protists is the product of their extreme evolutionary age, as some extant protist species exist for more than a billion years.

Based on extensive phylogenetic studies and modest fossil records, we can justifiably speculate that the molecular and cellular features of at least some protist lineages did not alter much during the eons they occupy the Earth. Indeed, some protists, such as marine heterotrophic flagellates, may be surprisingly similar to the LECA, or the last ekaryotic common ancessor from which all extant eukaryotes are derived. Hence, these tiny unicellular organisms encapsulate in themselves substantial fraction of the evolutionary history of Life on Earth.

While the species richness, as well as morphological and molecular features of these tiny critters may by many be considered just an academic problem, it is worth noting here that the invisible life supports the visible life, which would not survive without the former a minute. This is apparent from the fact that protists, mostly those occupying the upper layer of the world ocean, are responsible for the production of up to 50% of all planetary oxygen. These unicells are undoubtedly beneficial for the whole planetary ecosystem, and thus for us humans, yet there are also numerous protists that opted for

a parasitic way of life. They are responsible for a wide range of diseases, and those species that cause some devastating disease of humans logically belong to the best studied protists.

Here is where Prof. Francisco J. Ayala comes in. Whether his interest in these organisms was inspired by Saint-Exupery's Little Prince, who said "What is essential is invisible to the eye" or whether he simply wanted to help alleviating human suffering, we will probably never know, but it does not matter. As a man of many trades in science, Francisco liked to embrace challenges and studying parasitic protists was, especially in his time, a true challenge. As with other challenges in his life, he succeeded and soon became a prominent parasitologist.

Let us briefly acquaint with Francisco's main contributions to the field of parasitology, which spans approximately 40 years. Francisco's first paper on this subject appeared in 1987, when his laboratory was still fully engaged in the studies of genetic variations in *Drosophila*. It was likely an inspiration drawn from his work on this insect model, where he was mapping genetic variations using differential mobility of selected enzymes (Ayala, 1983). An application of this established technique to trypanosomes, namely to *Trypanosoma cruzi*, the causative agents of Chagas disease, enabled novel insight in its heterogeneity. Although he was more aware of the pitfalls and limitations of the molecular clock than most (Ayala, 1986), Francisco and coworkers were able to harness a lot of information from enzymatic mobilities, the results of which were published in a highly influential and widely cited paper on the multiclonal structure of the *T. cruzi* populations (Tibayrenc et al., 1986). Although a virtual newcomer to the field, he quickly cracked one of the hard nuts in the field, by showing that the extreme DNA sequence differences among various isolates represent a characteristic feature of *T. cruzi* and that this must become an important part of our thinking about the prevention, spreading, and natural foci of Chagas disease. While many ailments caused by parasitic protists have within the latest decades been suppressed due to the development of new drugs and epidemiological interventions, regrettably this is not the case of *T. cruzi*. With the estimates predicting ~10 million infected people worldwide, and the deadly parasite effectively spreading outside of Central and South America due to blood transfusion, the search for new measures to fight it is of utter importance.

The work with this trypanosome has lead Francisco and his long-term collaborator Michel Tibayrenc to formulate a Clonal Theory of Parasitic Protozoa (Tibayrenc, Kjellberg & Ayala, 1990), which turned out to be a truly conceptual breakthrough, with important biological and medical consequences.

While this treatise is not a proper ground for its detailed description, it shall suffice to say that the authors for the first time stated that sexual recombination is (exceedingly) rare in natural populations of parasitic protists. While it was ever since strongly supported by some authors and heavily criticized by others, it was repeatedly updated and extended, with a characteristically lucid language of Francisco (Tibayrenc and Ayala, 2012; 2013; Rougeron et al., 2009), as well as defended at numerous scientific meetings.

The particularly sophisticated killer *T. cruzi* was certainly a favorite object of Francisco among parasitic protists, but I and others succeeded to turn his attention to another band of widespread parasites – members of the genus *Leishmania*. In over 60 countries, they are responsible for a range of diseases under the umbrella term leishmaniasis, which range from a small skin ulcer, characteristic for the cutaneous form of the disease, to lethal organ failures, termed visceral leishmaniasis or kala-azar. In 2006 I was member of a consortium of a dozen European laboratories that generated a substantial amount of sequence data from a big collection of *Leishmania* isolates, but we were scratching our heads about how to interpret them. The leader of this Euroleish consortium, Michael Miles, suggested that I contact Francisco in this matter, which I did. After several in-person meetings and extensive communication “over the pond” with Francisco, we have not only described predominantly clonal population structure of these parasites worldwide, but also a hotspot in Somalia, where sexual recombination was way more frequent than elsewhere. Moreover, another important aspect of the study was our proposals as to how leishmaniasis spread around the world, primarily emerging from Central America (Lukeš et al., 2007). As a matter of fact, even 17 years later, most of our predictions still hold.

During the fruitful discussions with Francisco about these and related flagellates, we touched on a conundrum associated with trypanosomes that were found outside of Africa, causing deadly diseases surra and dourine in horses, camels, water buffaloes and occasionally other hosts, such as dogs. They were for over 100 years associated with species named *Trypanosoma equiperdum* and *Trypanosoma evansi*, but we both had a feeling that this is wrong and that these flagellates are just forms of *Trypanosoma brucei*, which causes the infamous sleeping sickness in humans and livestock across Africa. Hence, we have performed some assays to test our hypothesis, which turned out correct (Lai et al., 2008). Furthermore, we have shown that it was the loss of mitochondrial DNA that allowed these pathogens to lose their otherwise essential dependence on the tsetse fly vector, which is confined to Africa, and allowed them to spread to all other continents except Antarctica. This was

a hard sell to other scientists, because it was a unique case when a loss of DNA counterintuitively turned into a significant gain for its bearer (Lun et al., 2010). It was Francisco's eloquence that was able to convince the reviewers that we were correct. And I am pleased to say that eventually our view was not only accepted but further supported by whole-genome data obtained in the meantime.

My colleagues and I have joined forces with Francisco in several other stories (f.e. characterization of the first eukaryote living totally without heme—Kořený et al., 2012), but here I will describe just one more of our successful collaborations. It concerns an organism that we called *Paratrypanosoma confusum*, as it confused us all for quite some time. It puzzles researchers where does the long list of strange features for which trypanosomes are well known even outside of the field of parasitology come from? Is it because these protists developed a highly complex life cycle, or because they decided to occupy host's blood and thus expose themselves to a direct and permanent immune attack, or is it the product of an extremely long independent evolutionary path? Although we cannot turn the wheel of evolution and look millions of years back, one way of shedding light on this issue is to look for protists that are related to trypanosomes yet are either still free-living or still did not adopt their sophisticated parasitic life style. Did they already evolve (some) peculiar features of trypanosomes? If so, are these features less complex? If found, will these organisms be direct predecessors or just offshoots of the evolutionary tree that have little bearing on the forces that shaped human pathogens? In a typical serendipity of science, we found such a primitive trypanosome in midges sucking blood on birds. And it was again the deep knowledge of evolution that Francisco possessed that was instrumental for the interpretation of our findings (Skalický et al., 2017).

Towards the end of his life, Francisco affiliated himself with the University of South Bohemia, where I work for now a few decades. While he gave there a seminar on the transmission of malignant malaria from gorillas to humans, I felt that he decided to leave the next open questions of parasitology to the younger generation and was more concerned about the relationship between science and religion, a subject that will be comprehensively covered by others.

In closing, I can only speculate about what reason or motivation drew Francisco to devote a substantial part of his intellect to the study of parasitic protist, especially those plaguing the tropical and poor countries. Being a towering humanitarian, he probably simply wanted to help millions stricken by malaria, sleeping sickness, Chagas diseases and leishmaniases, and it is

beyond doubt that his research contributed to the fact that the incidence of most of these parasitoses are on decline. As one of the last true renaissance men of science, Francisco could have entered many other fields in biology and would certainly become prominent there, yet he decided to study microscopic parasites and I was fortunate not only to be close by but also enjoy the friendship with him and Hana Ayala.

Note: The literature has been confined only to papers co-authored by Francisco J. Ayala.

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