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Grassi *versus* Ross: who solved the riddle of malaria?

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Introduction

Malaria, or paludism, is a disease that has been the scourge of populations in tropical and temperate-hot areas of the world since antiquity. The Italian name, *malaria*, identifies the disease better than the French term, *paludisme*. In fact, it refers to *mala aria*, the bad air, the miasmas evaporating from the stagnant waters of marshes, which the ancients believed were the origin of the disease. It was not until the second half of the nineteenth century that scientists started to search for the microscopic "bug" that gives rise to the incurable disease. By then, adequate optical instruments for this purpose had finally become available, and Robert Koch (1843–1910) and Louis Pasteur (1822–1895) had laid the foundations for scientifically based clinical microbiology.

The reason why malaria became the focus of research was not the same for all of the individuals involved in the quest for its causal agent. France and the British Empire had expanded their colonies throughout regions of the world where malaria was the most serious and debilitating of the many parasitic tropical diseases, a factor that led to limited exploitation of natural resources and, accordingly, lower economic profits. Therefore, prominent scientists decided to tackle the problem of *paludisme* in France and in Great Britain. Malariological research also thrived in Italy, both from the clinical and the public health points of view. But Italian malarial studies had a different perspective. For French and British researchers, malaria was a "tropical disease", a "colonial" problem. For Italian physicians, it was a disease endemic to their country and a scourge that hindered the development of many southern areas of the country, which had achieved national unity only a decade earlier. Rome, the capital city of that young kingdom, was prey to malaria in the summer and autumn months.

Abstracted by **Mercè Piqueras**, INTERNATIONAL MICROBIOLOGY, from a lecture given by the author at the Institute for Catalan Studies, Barcelona, on October 19, 2005.

Colonial wars or domestic burden

Among the French and British scientists who carried out research on malaria, the most outstanding were Laveran, Patrick Manson, and Ronald Ross. Charles Louis Alphonse Laveran (1845–1922) was a military physician in Algeria when, in 1880, he described malarial parasites in the blood of patients during malarial fever episodes. He called this microscopic organism *Oscillaria malariae*. The discovery was first met with skepticism. However, when Laveran gave up his military career in 1896 and resumed his studies at the Institut Pasteur, he obtained new, indisputable evidence that was later confirmed by foreign researchers. Because of his discovery, Laveran is considered the father of protistological parasitology.

In 1879, Patrick Manson (1844–1922), a Scottish physician who had worked in China for over 20 years, demonstrated that so-called Bancroft's filaria (*Wuchereria bancrofti*) was transmitted via the bite of a mosquito of the genus *Culex*. He proposed that a mosquito of that genus should be also involved in malaria, but as part of a bizarre cycle: the parasites were released in water by dead mosquitoes and then transferred to humans when they drank the water. Manson corresponded extensively with Ronald Ross, including 173 letters (gathered in a book in 1998) in which he followed and steadily guided Ross's progress in the study of malaria in India.

Ronald Ross (1857–1932) is the main character in the history of British malarial research. Born in India, he joined the army as an officer of the Indian Medical Service. In 1897, when he studied malaria in birds, he described oocysts of the malarial parasite in the walls of the stomach of an unclassified mosquito ("a grey mosquito, a dappled winged mosquito"), which he thought was probably *Culex*. This was the starting point of Ariadne's thread that would eventually lead to the exit from the labyrinth of malaria.

A map of Italy published in 1882 indicated in red the areas with widespread malaria and in yellow the areas where the disease was present. The red area included vast coastal areas of Tuscany (Maremma), Latium (Roman plain and Pontine marshes), and Campania. Also at high risk of malaria were the Venetian lagoon areas, the Po River Delta, the Ionian Coast of Calabria, and the coasts of Sardinia and Sicily. Even more tragically amazing is another map, published in 1899, that was produced by a professor of hygiene, Augusto Celli, which indicated those railway lines where the risk of contracting malaria during a train trip was high! Italian physicians thus experienced malaria as a daily domestic tragedy, and were highly motivated to solve the mystery of the origin and transmission of the disease.

First malaria studies in Italy

The death from malaria of a nine-year-old boy in Rome at the turn of the twentieth century spurred several Italian physicians to carry out research aimed at solving the riddle of the cause of the disease. I will mention only a few eminent figures.

Ettore Marchiafava (1847–1935) and Augusto Celli (1857–1914), both from the Faculty of Medicine of Rome, believed that the pathogenic factor of malaria was a bacterium, *Bacillus malariae*. After careful studies, however, they agreed with Laveran and recognized the protozoan nature of the malarial parasite. They proposed the name *Plasmodium* for the protozoan identified and named by Laveran. In fact, in 1885, *Oscillaria* had been assigned to another organism, a "blue-green alga" (currently a cyanobacterium). Marchiafava and Celli also identified two species of *Plasmodium*, *P. falciparum* and *P. vivax*.

Camillo Golgi (1843–1926), Professor of General Pathology at the University of Pavia, in northern Italy, is well-known worldwide for his research in the physiology of the nervous system (he was awarded the 1906 Nobel Prize in Physiology or Medicine, together with Santiago Ramón y Cajal). Nevertheless, he was also deeply involved in malaria research. Indeed, residents of the agricultural areas along the Po River had a high risk of the disease, especially where there were extensive rice fields, such as the countryside around Pavia. Golgi made a notable contribution to malariology by relating the clinical sign of the fever episode with the schizogonic phase of the plasmodium, and by showing that the so-called tertian and quartan intermittent fevers are due to the presence in the blood of two different *Plasmodium* species (*P. malariae* and *P. vivax*), sometimes present together.

Last, but certainly not least, is Battista Grassi (1854–1925), the other main character in this story. Grassi (Fig. 1) was born in Rovellasca, a rural town not far from Milan. Even though he graduated in Medicine at Pavia, he felt driven to become a zoologist because of his frequent contact with nature during his childhood and adolescence and because the University of Pavia was, at the time, the "sun of Italian biology", as Grassi himself used to say. After graduation, he worked at the Naples Zoological Station, founded by Anton Dohrn (1840–1909), and at the Messina oceanographic station of Nicolaus Kleinenberg (1842–1897). His training was completed at the University of Heidelberg under the guidance of two great scientists: Carl Gegenbaur (1826–1903), who reorganized comparative anatomy in Darwinian terms, and Otto Bütschli (1848–1920), one of the greatest experts on protozoans. At a very young age, Grassi became Professor of Zoology at Catania and was already famous for two extensive monographs, one on the Chaetognatha and the other on the vertebral column of fishes.

Grassi and malaria

In 1888, Grassi began to study malaria in birds at Catania, in collaboration with the medical clinician Riccardo Feletti. In 1890, they published a monograph in which they described the malarial cycle in different species of birds, including owl, pigeon, and sparrow. It is here that we recognize Grassi's zoological approach to the problem, the constant physiognomy of Battista Grassi's method of investigation: different species of birds, belonging to different orders (Strigiformes, Columbiformes, and Passeriformes), are parasitized by different species of protozoans: only *Halteridium* in pigeons,

Fig. 1. The contender, Battista Grassi (1854–1925).

but also *Proteosoma praecox* (*Haemoameba*) in the sparrow. All this took place five years before Ross turned his attention also to the study of malaria in birds in India.

Grassi was appointed Professor of Comparative Anatomy at La Sapienza, in Rome, in 1895. By then, his international reputation was established, not so much for his work on malaria in birds, but for his epochal contributions to entomology and biological oceanography concerning the life cycle of the common eel. In 1896, The Royal Society of London awarded him the prestigious Darwin medal, which, as specified by the zoologist Edwin Ray Lankester (1847–1929) in a letter announcing the award and now preserved in the Grassi Archive at La Sapienza, rewarded "those naturalists who are still in active work and especially doing work which has an important and direct role bearing on Mr. Darwin's own investigations & theory". Before Grassi, the prize had been awarded to Alfred Russell Wallace (1823–1913), Joseph Dalton Hooker (1817–1911), and Thomas Henry Huxley (1825–1895). In the opinion of English zoologists, therefore, the value of Battista Grassi as a naturalist was equal to that of the three greatest and most faithful friends of Charles Darwin.

In Rome, Grassi met the group of Roman malariologists, who convinced him of the validity of the transmission of the plasmodium via a hematophagous insect, a hypothesis he had until then considered doubtful. The problem was to identify the insect responsible for transmission. Grassi approached his research with the tools of the zoologist, namely knowledge of the systematics of the group and of the geographical distribution of the species. On the basis of the epidemiology of malaria and the distribution of mosquitoes present in the malarial zones, he focused his investigations on three species suspected of malarial transmission, *Anopheles claviger* (synonym *A. maculipennis*) and two *Culex* species (but not including the common *C. pipiens*), and he communicated this result to the Lincei Academy on September 19, 1898.

On November 6, 1898, Grassi announced to the Lincei Academy that, with two colleagues, Drs. Bignami and Bastianelli, he had infected a volunteer by exposing him to the bite of these three mosquito species. Suspicion of the two *Culex* species faded immediately and the mosquitoes were acquitted of the crime of being vectors of the infection. On November 28, 1898, a formal note was sent to the academy and read in the academic session of December 4, 1898, where it was announced that a healthy man in a non-malarial zone had contracted tertian malaria after being bitten by an experimentally infected *Anopheles claviger*. The experimental phase ended on December 22 with a communication to the Lincei Academy that described the entire developmental cycle of the plasmodium in the body of *Anopheles claviger* and stated that it corresponded to what Ross had described for *Proteosoma* in *Culex pipiens* in the malarial cycle of birds.

The experiment had been conducted with exceptional rigor; the *Anopheles* mosquitoes were raised in the laboratory beginning from the larval stages and starved until they had bitten a patient who had semilunar bodies in his blood—the only stage that could have developed into gametophytes and thus triggered the gonochoric cycle in the body of the mosquito. A healthy person was then exposed to the bite of these mosquitoes in a place protected from the introduction of other mosquito species.

The spy that came in from the cold

The first issue of the Annales de l'Institut Pasteur of 1899 contained an article dated "Calcutta, 31 December 1898" by Major Ronald Ross (Fig. 2) and entitled "Du rôle des moustiques dans le paludisme". The insect responsible for transmission of the disease was indicated as "moustique d'une nouvelle espèce", just as in the note of 1897 it was indicated as a "grey" or "dappled winged" mosquito, which were absolutely invalid names for the Linnaean nomenclature. Ross was not a zoologist, however, and he completely lacked the tools of zoological systematics. At the margin of Ross' article in the issue of the Annales Pasteur, Grassi made many handwritten notes, including: "*non dice che fosse Anopheles*"

Fig. 2. The winner, Ronald Ross (1857–1932).

(he doesn't say that it was *Anopheles*). On June 4, 1900, the Memoirs of the Royal Lincei Academy published an article by Grassi entitled "Studies on Malaria by a Zoologist". It consisted of 200 large-format pages that summarized the research Grassi had carried out from 1896 to 1899. In the article, Grassi defined himself as a zoologist.

In the crucial years of Roman research on the transmission of malaria (between 1897 and 1898), the English physician Edmonston Charles visited Grassi's laboratory in Via de Pretis and those of the other malariologists at the Santo Spirito Hospital. The Italian scientists, flattered by the interest of an English colleague in their studies, greeted him without suspicion of his motives. He then reported to Ross the information obtained. When the polemic about the priority of the discovery of the insect responsible for malarial transmission emerged, Ross felt obliged to make public the letters received from Charles.

Charles wrote to Ross: "[…] I called on Dr. Manson before leaving London to get the latest news of what progress you had made in your work, in order to let the Italians know. They have been working in various directions this summer, but up till this week without being able to show any definite results. […] According to Grassi it would seem there are some fifty varieties of mosquitoes in Italy. Only six, however, seem to frequent these selected malarial positions. Besides the mosquitoes, the larvae were also brought up, and allowed to develop in Rome."

The Roman malariologists eventually became mistrustful of the Englishman and did not tell him the truth about their advancements. In fact, by November 4, 1898, the suspects had already been limited to just three species and the acquittal of the two *Culex* species had already been decided. In another letter, dated November 19th, Charles wrote to Ross: "I went to his [Grassi] laboratory to try and get him to give you a few specimens of the different kinds of mosquito. I did this under the impression that he had completed his investigations. He told me, however, they were far from complete, and did not give me the specimens."

Charles' initial impression was right. By then, Grassi, Bignami, and Bastianelli had actually identified the malarial vector, and on December 4th, only two weeks after Charles' visit, they published their finding in the Reports of the Lincei Academy. Charles' letter then continues with an interesting sentence: "He [Grassi] spoke in the highest terms of praise of your work; he has your first report [the note of December 18, 1897 in the *British Medical Journal*], and told me to write to try and get your future reports at an early date for him."

On that date, therefore, the relationship between the two scientists was one of mutual respect, confirmed in a letter dated "Calcutta 5 February 1899" that Ross sent to Charles: "My dear Dr. Charles, very many thanks for your last letter with the translation of Grassi, Bignami, and Bastiamelli's note. This is good indeed. Pray give them my congratulations. […] I thought that the grey mosquito is *Culex pipiens*, but was not quite sure. Of course there is a whole family of allied grey mosquitoes."

This friendly and collaborative climate continued in the spring–summer of 1900, when Patrick Manson organized a crucial experiment to be conducted in an Italian malarial zone. A small building, in the style of English hunting huts, was designed and built in England and then assembled in Italy, in the Castelfusano pinewood on the hunting estate of the kings of Italy, which was near Ostia, not far from Rome. Two intrepid doctors, the Italian Luigi Sambon and the Englishman G.C. Low, both of the London School of Tropical Medicine, one of the most prestigious centers for the study of tropical diseases at the time, dwelled in this "mosquito-proof" hut over the period of the summer–autumn fevers, which was also the period of maximum reproductive activity of the mosquitoes. The doctors, and their servants, remained free of malarial infection after a stay of three months. Bastianelli and Grassi followed the experiment, and the latter sent Manson a telegram dated September 13, 1900: "Assembled in British mosquito proof hut having versified (*sic*) [instead of "verified"] perfect health of experimenters among malaria stricken inhabitants. I greet Manson, who first formulated mosquito malaria theory."

The shadow of the Nobel Prize

At the end of 1900, Ross began a defamatory campaign against the three Italian researchers to claim priority of discovery of the mechanism of transmission of malaria, surely with the prospect of the Nobel prize in mind. He questioned the originality of Grassi's research, insisting that his indication that a "grey mosquito with dappled wings" was responsible for the transmission had guided Grassi in the identification of the vector. He even accused Grassi of fraud, on the basis of incorrect dating of the notes presented to the Lincei Academy, which instead had been precisely certified by the date of presentation in the Academy's public session. Grassi reacted harshly to these accusations, which he felt offended his honor as a scientist.

The Swedish Academy of Sciences had shown an intention to award the Nobel prize of Physiology or Medicine in recognition of this discovery of enormous importance for global public health, and was therefore very embarrassed by the dispute. In response, it appointed as "neutral" arbitrator a scientist of great authority and expertise in the specific field: Robert Koch, who had carried out research on malaria in the spring of 1898 at Grosseto, the main town of the Tuscan Maremma, a malaria region par excellence. On that occasion, he had argued with Grassi, who had disagreed with some of the German microbiologist's analytical methods and deductions. The logical consequence was that Koch's arbitration was in favor of Ross, who received the prize in 1902.

The dispute between Grassi and Ross about the priority of discovery has been interpreted as motivated by personal ambition, national pride, the desire for academic pre-eminence, and similar psychological and sociological explanations that have very little to do with science. In this regard, in 1998, Bynum wrote that the dispute "[…] is one of the least attractive episodes in the whole history of malariology," and this may be partly true; both scientists had strong personalities, and it was not easy to find a point of agreement. It might have been objected that, all in all, to have attributed a Linnaean name to the insect was a marginal part of the problem, but if this might have been justified in the nineteenth century it was no longer so at the threshold of the twentieth century. This is the unanimous judgment of science historians, who now attribute equal merit to both scientists.

To each his own

The true meaning of the dispute, though, reflects the different ways of tackling problems in biological research, in this case, parasitological cycles. Grassi's approach was the one characteristic of zoological research: systematic, comparative, experimental. In contrast, Ross' approach was empirical and intuitive. Medicine in the 1800s, but also afterwards, up until recent times, was an empirical science. Not so zoology, which the Darwinian revolution approached with positivistic concreteness. For a post-Darwinian zoologist, the question of species was the focal point of the process: an animal remained undefined until it was placed in a context, no longer merely a classificatory context, but also an evolutionary one. For Grassi, nomenclatural meticulousness was almost an obsession.

The second tool that guaranteed Grassi's success was the comparative approach, which he thought was the main route to arrive at the answer of the malaria question. In his research, he excluded species that could not be malarial vectors and unequivocally identified *Anopheles claviger* (syn. *A. maculipennis*) as the only vector of malaria in Italy. For Grassi, parasitology was a zoological science; it was the application of Darwinism to pathology.

Grassi also had his dogma: "There is no malaria without *Anopheles*". The opposite, however, was not always the case: he noticed that there were areas where *Anopheles* was abundant but malaria was absent. An initial hypothesis, expressed in the second edition of his article, was to relate this phenomenon to the thermophily of the *Anopheles* mosquito. In areas with cold nights, the mosquito did not fly and bite humans, but stayed in the warmer stalls to bite livestock.

Eradication of malaria in Italy

After World War I, malaria flared up with renewed vigor. Mortality from malaria had rapidly decreased in Italy starting from 1898, after discovery of the vector and institution of zooprophylactic activity, as well as the free distribution of quinine. As a result, in 1915, the number of deaths had decreased from 600 per million inhabitants to less than 50; however, it then increased to 320 per million in 1919 (Fig. 3). Resuming his research, Grassi turned again to the problem of "anophelism without malaria". He identified three localities with a typical malarial environment, all infested by *Anopheles maculipennis* but not affected by malaria. In 1921, he demonstrated that "there is certainly a biological race of *Anopheles* mosquitoes that does not bite man."

A year after Grassi's death in 1925, one of his pupils showed, on the basis of these observations, that there are six species of *Anopheles* in the *maculipennis* complex, which are indistinguishable except for their egg morphology. Of these six "new" species, *Anopheles labranchiae* and *A. sacarovi,*

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Fig. 3. Argentinian leaflet for an antimalaria campaign.

present in highly malarial zones, mainly bite humans, while the typical form of *A. maculipennis* only bites animals. Another species, *A. messae*, mostly bites animals, but sometimes also humans. These findings demonstrated the importance of precise systematic identification of the vector in antimalarial prophylaxis; rough identifications like “grey mosquito” or “dappled winged mosquito” could not suffice. It is interesting that complexes of *Anopheles gambiae* and *A. arabensis*, the two species most responsible for malarial transmission in the world, also show anthropophily and zoophily, characterizing different populations of the two species.

Malaria was eradicated in Italy through systematic control of the insect vector. This was conducted on two fronts: reclamation of marshy environments, and direct biological and chemical control of the mosquito. Although the former involved hydraulic engineering projects, the latter mainly required the work of zoologists, direct and indirect pupils of Grassi and Bignami who continued their work.

Coda

Malaria still claims many victims throughout the world—far too many in countries that, like Italy at the end of the 1800s, cannot proceed on the road to development until the disease has disappeared. Is the Italian model applicable to such countries? The problem is certainly much more complex; the vastness of the areas affected, sometimes whole continents or subcontinents, and the ecological and socio-economic situations hinder antimalarial campaigns. New strategies of vaccination or of genetic engineering aimed at creating transgenic *Anopheles* mosquitoes are being attempted. Therefore, there arises once again the need for genetic characterization of *Anopheles* populations—a task requiring that zoologists and geneticists work together.