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Battista Grassi: a zoologist for malaria*

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Malaria is probably one of the oldest diseases, and it has been the scourge of populations in tropical and temperate-hot areas of the world since antiquity. It is also known by its French term, *paludisme* although the Italian name, *malaria*, more accurately describes the disease. The Italian term refers to *mala aria*, bad air, i.e., 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 agent that gave rise to malaria. By then, optical instruments appropriate 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.

Historically, malaria has accompanied humankind for millennia. Alexander the Great and Dante Alighieri are among the most famous victims of this disease. Marcus Terentius Varro (116–27 BC) wrote "*Advertendum etiam siqua erunt loca palustria, et propter easdem et quod arescun crescunt, animalia quaedam minuta quae non possunt oculi consegui, et per aera intus in corpora per os ac nares perveniunt atque efficiunt difficile morbo*" (*De re rustica, libro* I) ("We must warn that marshy localities –when they dry out– allow the generation of animals so small that they are not visible, but which penetrate into the body through the air, passing through the mouth or the nose, thereby causing serious diseases"). Later, the Roman physician Giovanni Maria Lancisi (1654–1720) proposed that small worms (*vermiculi*) originating from marshes developed into noisy mosquitoes (*stridulos culices*) able to inject a poisonous liquid through the bite wound (*veneficum liquidum ifusum per vulnus)*.

In the second half of the nineteenth century, the colonial expansions of the scientifically advanced European powers were in full swing, especially of France in North Africa and of the British Empire in Asia. In those parts of the world, malaria was without doubt 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. Of these, the three who were the most important for our story are described.

Charles Louis Alphonse Laveran (1845–1922) was a military physician in Algeria when, on 6 November 1880, at the age of 33, he described malarial parasites in the blood of patients during malarial fever episodes (Laveran, 1880, 1881). He called this microscopic organism *Oscillaria malariae*. The discovery was initially met with skepticism; however, soon afterwards Laveran resumed his studies at the Institut Pasteur (he abandoned his military career in 1896), where he produced new, indisputable evidence, later confirmed by foreign researchers, of the link between the blood parasite and the disease. Laveran's discovery would eventually lead to his recognition as the father of protistological parasitology.

Sir Patrick Manson (1844–1922) was a Scottish physician who worked in China for over 20 years. In 1879, Manson demonstrated that 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 was also involved in

Figure 1. Ronald Ross (1857-1932) (Drawing by Diego de Merich).

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malaria, but as part of a bizarre cycle: the parasites were released in water by dead mosquitoes and then transferred to humans who drank the water. Manson had a rich correspondence with Ronald Ross, consisting of 173 recently published letters (Bynum & Overy, 1998), in which he followed and steadily guided Ross's progress in the study of malaria in India.

Sir Ronald Ross (1857–1932) (Figure 1) is a very important character in the history of malarial research and thus in our story. Born in India, the son of a general in the British imperial army, he entered the army as an officer of the Indian Medical Service. In 1897, during his studies on malaria in birds (Ross, 1897a, b), 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 is the starting point of Ariadne's thread that would eventually lead to an exit from the labyrinth of the origins of malaria.

Malaria in Italy

Malaria research also thrived in Italy, especially from the clinical and public-health points of view. But the perspective of Italian malarial studies differed from that in France and Great Britain: for French and British researchers, malaria was a "tropical disease", a "colonial" problem. However, for Italian physicians, it was a disease endemic to their country, a scourge that hindered the development of many southern areas of a country that had achieved national unity only a decade earlier. Rome, the capital city of this young kingdom, was prey to malaria in the summer and autumn months.

In 1890, Giustino Fortunato (1848–1932), one of the leaders of the Italian public-health movement, was native of Rionero, in Volture, a malarial zone in southern Italy. Fortunato maintained that "[…]*se la malaria proviene del suolo, la nostra lotta contro la malaria è vana. Siamo nati in un suolo non benigno e contro i fati è vano lottare*", i.e., if malaria comes from the soil, our fight against malaria is hopeless. We were born on a malevolent soil and it is hopeless to fight against fate." In a famous discourse in Parliament, he warned the government that until southern Italy was rid of the debilitating disease, there could be no development of the region, A map of Italy published in 1882 indicated in red the areas with widespread malaria and in yellow the areas where the presence of the disease was limited. 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 and Po River delta, the Ionian Coast of Calabria, and the coasts of Sardinia and Sicily. Even more tragically amazing was a map published in 1899, by the Professor of Hygiene Augusto Celli (Celli, 1899), which indicated those railway lines where the risk of contracting malaria during a train trip was high!

The drama of malaria in Italy, however, was ancient. The Greek geographer Strabo (63 BC–21 AD ca.) wrote about Latium "*Omne Latium felix est et omnium rerum ferax, exceptis loci quae palustria sunt atque morbosa*" (Geographia, libro V). The popes and the entire pontifical Court escaped from Rome in the summer months and only returned at the end of autumn. On the initiative of Cardinal Juan de Lugo (1583–1660), the powdered bark of the Peruvian cinchona (*Cinchona succirubra*) was first used as a remedy for the recurrent malarial fevers in the ancient hospital of Santo Spirito, in Sassia in Rome (Dobson, 1999).

The Grassi Archive, located in the Department of Animal and Human Biology [(Capanna & Mazzina, 1998); cited in the text as *A.G., DBAU*], contains the following report among the clinical records of a malaria investigation conducted in Rome and the surrounding countryside by Grassi and collaborators in 1900. This report [*A.G., DBAU . Sec.003 Malaria; Box 11, file1*] (Capanna, 1996a) is disturbing in its laconic description of tragic events: "R. A., from Anguillara (Rome), 9 years old. Fever on 2 August […] suffered a severe bout of fever on 22 August, and died of pernicious algid fever on the night of 23 August in Santo Spirito Hospital."

In Rome, at the beginning of the twentieth century, a nine-year-old boy died of malaria within a period of 20 days. This was the potent stimulus that prompted a group of Roman doctors to resolve the problem of malaria. Of these physicians, I will mention only a few eminent figures.

Ettore Marchiafava (1847–1935), Professor of Pathological Anatomy, and Augusto Celli (1857–1914), Professor of Hygiene, taught in the Faculty of Medicine of Rome, where they collaborated closely in their research on malaria. They were somewhat perplexed by Laveran's discovery, since they believed that the pathogenic factor of malaria was a *Bacillus*, *B. malariae*. However, after careful studies, they agreed with the observations of the Frenchman indicating a protozoan as the malarial parasite (Marchiafava & Celli, 1883a, b). Since the generic name *Oscillaria* proposed by Laveran was occupied by another organism, a green-blue filamentous alga (Cyanophycaea), in 1885, the two scientists proposed the name *Plasmodium* for the genus (Marchiafava & Celli, 1885).

Amico Bignami (1862–1929), Professor of General Pathology, was a pupil of Marchiafava and together with Giuseppe Bastianelli (1862–1959), clinician-physician, collaborated closely with Grassi in the identification of the malarial vector, especially with regard to its intrahematic cycle outside the human body (Bignami, 1896, 1898; Bignami & Bastianelli, 1898) and to its clinical aspects. An important contribution of Marchiafava and Bignami (1894) was the identification of two species of *Plasmodium*, *P. falciparum* and *P. vivax*

Camillo Golgi (1843–1926), Professor of General Pathology at the University of Pavia, was awarded the Nobel Prize in 1906 for his contributions to the physiology of the nervous system, but he was also deeply involved in malaria research. Indeed, 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 malaria studies (Golgi, 1886, 1889) 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*), which are sometimes present together.

And last, but not least, there is Grassi (1854–1925).

Grassi's studies on malaria

Gianni Battista Grassi (Figure 2) was born in Rovellasca, a rural town not far from Milan. He was a country boy who spent his childhood and adolescence immersed in nature –a way of life that led to his training as a zoologist (Capanna, 1995). He graduated in Medicine at Pavia, a university that was, at the time, the "sun of Italian biology", as Grassi used to say, since Golgi, Perroncito, and Bizzozzero, the great masters of nineteenth century cellular physiology, taught there. But his vocation was zoology. Thus, 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 "German" 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 large monographs, one on the Chaetognatha (Grassi, 1883a) and the other on the vertebral column of fishes (Grassi, 1883b).

Figure 2. Battista Grassi (1854-1925) (Drawing by Diego de Merich).

In 1888, Grassi began to study malaria in birds at Catania, in collaboration with the clinician Riccardo Feletti, and in 1890 he published the monograph "*Ueber die Parasiten der Malaria*" in *Zentralblatte für Bakteriologie und Parasitenkunde* (Grassi & Feletti, 1890, 1891a, b), in which he described the malarial cycle in different species of birds, such as owl, pigeon, and sparrow. It was here that Grassi's zoological approach to the problem and his consistent investigations into the pathogenesis of the disease become evident. Grassi found that different species of birds, belonging to different orders (Strigiformes, Columbiformes and Passeriformes), are parasitized by different species of protozoans: in pigeons, only by *Halteridium*, and in the sparrow only by *Proteosoma praecox* (*Haemoameba*). All this took place 5 years before Ross turned his attention to the study of malaria in birds in India.

In 1895, Grassi was appointed Professor of Comparative Anatomy at La Sapienza, in Rome. By then, he was also famous overseas, not so much for his work on malaria in birds, but for his epochal contributions to entomology (Grassi 1885, 1888a, 1888b, 1889, 1890, Grassi & Sandias 1893) and biological oceanography concerning the life cycle of the common eel (Grassi 1896a, 1896b; Grassi & Calandruccio 1892, 1895, 1897a, 1897b).

The Royal Society of London awarded him the prestigious Darwin medal for 1896 which, as specified by the zoologist Edwin Ray Lankester (1847–1929) in a letter announcing the award and now preserved in the Archive [*A.G., DBAU . Sec.001 Corrispondenza; Box 2, file7*], 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". The letter noted that, before Grassi, the prize had been awarded to Alfred Russell Wallace (1823–1913), Joseph Dalton Hooker (1817–1911), and Thomas Henry Huxley (1825–1895) (Capanna, 1996b). In the opinion of the English zoologists, therefore, the value of Battista Grassi as naturalist was equal to that of the three greatest and most faithful friends of Charles Darwin.

In Rome, Grassi came into contact with the group of Roman malariologists, who convinced him of the validity of the transmission of the plasmodium via a hematophagous insect, an hypothesis he had until then considered doubtful. The problem was to identify the incriminated insect with certainty. Grassi began the investigation with the tools of the zoologist, namely, knowledge of the systematics of the group and of the geographical distribution of hematophagous species (Ficalbi, 1896). On the basis of the epidemiology of malaria and the distribution of the mosquitoes present in malarial zones, he initially singled out 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 (Grassi, 1898b).

On November 6, 1898, Grassi announced to the Lincei Academy that, in collaboration with Bignami and Bastianelli, he had infected a "volunteer" by exposing him to the bite of these three mosquito species (Grassi, 1898c). Suspicion of the two *Culex* species faded immediately, and on November 28th a formal note was sent to the academy, and was read in the academic session of 4 December 4, 1898. In this note Grassi and his colleagues reported that a healthy man in a non-malarial zone had contracted tertian malaria after being bitten by an experimentally infected *Anopheles claviger* (Bastianelli, Bignami & Grassi, 1898). On December 22nd, in a second communication sent to the Lincei Academy (Grassi Bastianelli & Bignami, 1899), the entire developmental cycle of plasmodium in the body of *Anopheles claviger* was described. In addition, it was stated that the findings corresponded to what Ross had reported for *Proteosoma* in *Culex pipiens* in the malarial cycle of birds.

The experiments of Grassi and his coworkers were 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 whose blood contained "semilunar bodies", representing the only stage that could have developed into gametophytes and thus trigger 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 Grassi Archive includes documents relating to this scrupulous experimental procedure, such as the various notes addressed to Bastianelli, who was responsible for the mosquitoes, as well as the records of expenses, e.g. payments to the "volunteers" who were bitten, in which it appears that those patients with "semilune" (crescents) in their blood, i.e., the pre-gametocyte stage, were rewarded with more than two liras, twice the amount given to patients who had other stages of the parasite in their blood .

In the first issue of the *Annales de l'Institut Pasteur* of 1899, there appeared an article dated "Calcutta, 31 December 1898" authored by Major Ronald Ross and entitled "*Du rôle des moustiques dans le paludisme*" (Ross, 1899) 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, absolutely invalid names for the Linnaean nomenclature. However, Ross was not a zoologist and he completely lacked the tools of zoological systematics. Grassi correctly noted in the margin of Ross's article "he doesn't say that it was *Anopheles*!". The volume of the *Annales Pasteur* in our library contains numerous handwritten notes by Grassi (an angular calligraphy that reveals a punctilious character!).

On June 4, 1900, an article by Battista Grassi, entitled "Studies on Malaria by a Zoologist", was published in the Memoirs of the Royal Lincei Academy (Grassi, 1900). The article consisted of 200 large-format pages in which Grassi summarized the four years of research, from 1896 to 1899, and underlined the originality of his contribution by defining himself as a zoologist.

A spy story

At this point, we can introduce a little-known tale that might be called a "spy story". During the crucial years of research in Rome on the transmission of malaria (between 1897 and 1898), an English physician, Dr. Edmonston Charles, visited Grassi's laboratory in Via de Pretis and the laboratories of the other malariologists at Santo Spirito Hospital. He was greeted without suspicion by the Italian scientists, who were flattered by the interest of an English colleague in their studies. Dr. Charles then reported the information he obtained to Ross. 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 Dr. Charles. A rare publication by Ronald Ross, entitled *Letters from Rome on the New Discoveries on Malaria* (Ross, 1900), is in the Grassi Archive [*A.G., DBAU . Sec.003 Malaria; Box 12, file12, d.3*], and several passages from two letters that well-characterize the facts are quoted in the following.

In a letter dated November 4, 1898 (page 1), Dr. 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 Italian know. They have been working in various directions this summer, but up till this week without being able to show any definite results. Bignami has collected mosquitoes from four very malarious localities.*

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.

Two interesting observations emerge from these lines: that the game involved a triangle that consisted of Sir Patrick Manson in addition to Ross and Charles. In this well-orchestrated trio, the conductor, to stay within the musical metaphor, was Manson, the principal theme was played with virtuosity by Ross, and the basso continuo by Dr. Charles. Nevertheless, the Roman malariologists soon became mistrustful towards Charles and thus did not tell him the complete truth. In fact, on November 4th, the mosquito suspects had already been limited to just three species and the innocence of the two *Culex* species had already been decided. It is noteworthy that Charles wrote "varieties" and not "species", as a good zoologist would have done!

In another letter, dated November 19th (page 3), Charles wrote to Ross:

[…] *As, doubtless, it would help you to have named specimens of mosquitoes spoken of by Grassi, I went to his 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 correct. In fact, Grassi, Bignami, and Bastianelli had by that point identified the malarial vector and on December 4th, only two weeks after the visit by Dr. Charles, they published their success in the Reports of the Lincei Academy. The 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 18 December 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, as confirmed in a letter dated Calcutta, February 5, 1899 that Ross sent to Charles, who had sent him the English translation of the note by Grassi, Bignami, and Bastianelli of December 22, 1898 (Grassi, Bastianelli &Bignami 1899). Ross's letter states:

"*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 felicitation.*

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*." [*A.G., DBAU . Sec.003 Malaria; Box 12, file12*] (Capanna 1996b).

This friendly and collaborative climate continued in the spring and summer of 1900, when Patrick Manson organized a crucial experiment to be conducted in an Italian malarial zone (Manson, 1900). 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, near Ostia. This "mosquito-proof" hut was inhabited by two "intrepid" doctors, the Italian Luigi Sambon and the Englishman G.C. Low, both of the London School of Tropical Medicine, during the period of the summer-autumn fevers, which is also the period of maximum reproductive activity of the mosquitoes. The doctors (and their no less "intrepid" servants), remained free of malarial infection after a stay of three months (Sambon & Low, 1900, 1901). The experiment was followed by Bastianelli and Grassi, 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 salute Manson who first formulated mosquito malaria theory"* (quoted in Fantini 1999).

Who deserved the Nobel Prize?

At the end of 1900, Ross began a defamatory campaign against the three Italian biologists to claim priority of discovery for the mechanism of transmission of malaria–clearly with the possibility of winning the Nobel Prize in mind. He even put the originality of Grassi's research in doubt, maintaining that Grassi was guided in his identification of the vector by the fact that he (Ross) had already indicated that a "*grey mosquito with dappled wings*" was responsible for the transmission. Ross also accused Grassi of fraud on the basis of incorrect dating of the notes presented to the Lincei Academy; however, the dates had been precisely certified by the date of presentation in the Academy's public session. Grassi reacted harshly to the accusations, which in his opinion offended his honor as a scientist. The Swedish Academy of Sciences, which in effect had shown its intention to award the Nobel Prize for Medicine in recognition of this discovery of great importance for global public health, was very embarrassed and appointed as "neutral" arbitrator a scientist of great authority and expertise in the specific field, Prof. Robert Koch. In fact, Koch had carried out research on malaria in the spring of 1898 at Grosseto, the main town of the Tuscan Maremma, i.e., malaria country *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 was awarded the Nobel Prize for Physiology or Medicine in 1902.

The dispute between Grassi and Ross about the priority of discovery is usually interpreted as motivated by personal ambition, national pride, the desire for academic pre-eminence, and similar psychological and sociological positions that have very little to do with science. In this regard, Bynum (1998) wrote that the dispute "[…] is one of the least attractive episodes in the whole history of malariology" , and this may be true; both scientists had strong personalities and it was not easy to find a point of agreement. Although relating malarial transmission in birds to a haematophagous dipteran, albeit not systematically classified, was by itself a huge success for science, deserving of the Nobel Prize, precise identification of the nematocerous dipteran that transmitted malaria in humans must be considered a scientific success of equal importance. It might be argued that, all in all, to have attributed a Linnaean name to the culprit insect was a marginal part of the problem, but while this might have been justified in the nineteenth century, it was no longer so at the threshold of the twentieth. This is now the unanimous judgment of science historians, who attribute –now that the dispute has ended– equal merit to both scientists (Dobbel, 1925; Fantini, 1998; Dobson, 1999).

The true nature of the dispute, though, was the different manner of tackling problems in biological research, in this case, parasitological cycles. Grassi's method was characteristic of zoological research: systematic, comparative, experimental. The method pursued by Ross was empirical and intuitive (Fantini, 1999). Medicine in the 1800s, but also afterwards, until recent times, was an empirical science, but not so zoology, which with the Darwinian revolution tended toward a positivistic concreteness. For a post-Darwinian zoologist, identification of the 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. In 1899, while preparing to write the article "Studies on Malaria by a Zoologist", he needed an opinion about the nomenclature of the parasitic protozoan of malaria. He therefore turned to the leading expert on sporozoans, Prof. Raphaël Blanchard (1857-1919), whose return letter, dated November 9, 1899, is in the Grassi Archive [*A.G., DBAU . Sec.001 Corrispondenza; Box 1, file2*]. After consulting with various colleagues, including Alphonse Laveran, Blanchard provided a scheme of accepted names and different synonyms for *Plasmodium malariae*.

The comparative method was the second tool that guaranteed Grassi's success. It should be noted that Grassi learned the method from Carl Gegenbaur, one of the greatest post-Darwinian comparative anatomists, and the biological discipline he taught at the University of Rome was Comparative Anatomy. The comparative method was wisely used by Grassi not only in comparisons between species, but also between environments and ecosystems, and between species and the environments they inhabited. Thus, in a dialectical process, Grassi excluded those species that could not be malarial vectors and unequivocally identified *Anopheles claviger* (syn. *A. maculipennis*) as the sole vector of malaria in Italy. He wrote: "In medical science, the comparative method must be considered the main route to arrive at the solution of the problem." For Grassi, parasitology was a zoological science; it was the application of Darwinism to pathology.

Lastly, Grassi's experimental method must be appreciated.

The notion of progress through experience was deeply rooted in the tradition of the *Studium* of Pavia, the *Alma Ticinensis Mater* of Lazzaro Spallanzani (1729–1799), father of experimental biology. In 1782, Spallanzani had stated "*Lo sperimentare comunque è mestiere di tutti, lo sperimentare a dovere è sempre stato, e sarà sempre, di pochi*", i.e., "to experiment is the work of everyone, to experiment properly is, and always will be, the work of the few". Grassi certainly knew how "to experiment properly", as evidenced by his meticulousness in designing the experiments involving infection of mosquitoes born in the laboratory, using patients selected according to the hematic stage of the parasite and the subsequent biting of healthy patients in a protected environment. This approach was no doubt suggested by the cautious and scrupulous methods of Lazzaro Spallanzani.

Grassi's zoologist's method had already brought him success, for example, in the interpretation of various complex cases of human parasitoses related to the cycle of helminths. It is sufficient to cite his analysis of the cycle of *Ancylostoma*, which he published when he was still a student (Grassi and Parona, 1878), and especially his study of *Hymenolepis nana*, a cestode that, at the time, was ambiguously thought to have, or not have, an intermediate host. This may have been satisfactory state of information for a pathologist or a clinician, but not for a zoologist. The singularity of a species corresponds to the peculiarity of its reproductive cycle, both deriving from the species' unique evolutionary history. In fact, Grassi demonstrated *H. nana* to be a complex of two species: *H. nana* sensu stricto *,* without the intermediate host, and *H. diminuta*, which requires two hosts to complete its cycle (Grassi, 1887, 1888a).

Very similar to the case of *Hymenolepis*, on account of the nature of its zoological context and the comparative zoological approach with which it was tackled, was the question of "anophelism without malaria" (Fantini 1994). Grassi dealt with this problem, but did not have time to resolve it completely, even though he deduced those aspects related to the anthropophily and zoophily of several varieties of *Anopheles maculipennis*. Grassi also had his dogma: "There is no malaria without *Anopheles*"; but, already in 1899, at the time of the conclusive results concerning *Anopheles claviger*, he observed that there were areas where *Anopheles* was abundant but malaria was absent. A first hypothesis in this regard, expressed in the second edition of his article (Grassi, 1901), was to relate this phenomenon to the thermophily of the *Anopheles* mosquito. In areas with cold nights, the mosquito could not fly and bite humans, but was restricted to the warmer stalls, where it could bite livestock.

Vae victis!

After the disappointment of being excluded from the Nobel Prize, Grassi decided to stop studying malaria and to instead devote his attention to agrarian entomology and the metamorphosis of eels. However, the social importance of malaria studies convinced him to resume them in 1918. Indeed, after World War I, which had led to abandonment of the fight against malaria in favor of fratricidal fighting, malaria had flared up with renewed vigor. Mortality from malaria had rapidly decreased in Italy starting in 1898, after discovery of the vector and zooprophylactic activity, and due to the free distribution of quinine. By 1915, the number of deaths had decreased from 600 per million inhabitants to less than 50. However, in 1919, mortaility increased to 320 per million. 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: Orti di Schito, near Naples; Massarossa, in the Tuscan Maremma, near Lucca,; and Alberane, in the rice fields around Pavia. In 1921, he demonstrated that "there is certainly a biological race of Anopheles mosquitoes that does not bite man" (Grassi, 1921a, 1921b). 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, and they are indistinguishable except for their egg morphology (Falleroni, 1926). Of these six "new" species, *Anopheles labranchiae* and *A. sacarovi,* present in highly malarial zones, mainly bite humans, while the typical form of *A. maculipennis*, present at Orti di Schito, only bites animals. The species present at Massarossa in Maremma, *A. messae*, mostly bites animals, but sometimes also humans. Therefore, precise systematic identification of the vector is of obvious importance for the management of antimalarial zooprophylaxis, and rough identifications like *"grey mosquito"* or *"dappled winged mosquito"* are simply not adequate. It is interesting that the complexes of *Anopheles gambiae* and *A. arabensis*, the two species most responsible for malarial transmission in the world, also show anthropophily and zoophily in different populations of the two species.

Malaria has now been eradicated in Italy through systematic control of the insect vector. This has been conducted on two fronts: the reclamation of marshy environments, and direct biological and chemical control of the mosquito. Although the former aspect involved hydraulic engineering works, the latter mainly required the work of zoologists, direct and indirect pupils of Grassi and Bignami who have continued their work. Nevertheless, malaria has yet to be eradicated throughout the world. It still claims many victims, 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 of malaria is certainly much more complex today than it was in Grassi's time: the vastness of the affected areas –sometimes whole continents or subcontinents– and their ecological and socioeconomic situations pose serious impediments to antimalarial campaigns (Figure 3). New strategies of vaccination and genetic engineering approaches aimed at creating transgenic *Anopheles* mosquitoes are being attempted. Therefore, once again there is a need to genetically characterize *Anopheles* populations, and this work requires the services of zoologists and geneticists.

Figure 3. Argentinian leaflet for antimalaria campaign

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For further reading on the historical aspects and current topics of malarial research, I can point out 3 recent "special issues" of the journal Parassitologia:

W. F. Bynum & B. Fantini (Eds.) Strategies against malaria. Eradication or control? Parassitologia vol.40 (1,2) 1998, pp. 246. (Proceedings of the conference held at Annecy, France, April 17-21, 1996).

M. Coluzzi & D. Bradley (Eds.) The Malaria Challenge after one hundred years of malariology. Parassitologia vol 41 (1-3) 1999, pp. 528 + vi (Papers from the Malariology Centenary Conference, Accademia Nazionale del Lincei, Roma 16-19 November 1998).

M. Dobson, M.Malowany, D. Stapleton (Eds.) Dealing with malaria in the last sixty years.Parassitologia vol 41 (1, 2) 2000, pp. 182 (Proceeding of a Rockfeller Foundation Conference, New York 11-14 May, 1998).

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