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Review

Of parasites and men

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ABSTRACT

The living world has evolved and is evolving through interspecific relationships between organisms. The diversity of these interactions is enormous going from mutualism to parasitism. Humans live with a multitude of microorganisms, essential for their biology. However, interactions are not always advantageous. Indeed, many organisms might become pathogens, such as the *Plasmodium* species, the causative agents of malaria. Like many other microorganisms, they are «Machiavellian» in their capacity to elaborate a range of reproduction strategies, giving them a huge advantage in terms of adaptation. Here, we discuss the role played by parasites in the ecology and evolution of living organisms and particularly of humans. In the study of infectious diseases, humans are legitimately the focal point, although they represent only one ecosystem among many others and not taking this into account certainly biases our global view of the system. Indeed, we know only a minimal fraction of the microorganisms we live with. However, parasites have shaped and are still shaping the human genome. Several genetic signatures are the proofs of the selection pressures by parasites that humankind has endured during its evolution.

But, ultimately, what are the solutions for living with pathogens? Should we eradicate them or should we learn how to control and manage them?

Contents

Living organisms have tried, since their appearance, to fully exploit the environment (earth, water and air) that has been offered to them following the modification of the physico-chemical properties of Earth. Human beings are just one of such organisms, but they try to colonise everything as they now envisage the conquest of other planets as well. One of the downside of this desire of colonisation is the exploitation of life forms by other organisms, i.e., what nowadays is defined as symbiotism in the broadest sense.

However, these interspecific, tight relationships are not limited to parasitism. Indeed, the term of parasite or pathogen is used only for organisms that exploit their host, or in other words, that cause physical, physiological and other damages to the host. The world of such interactions is far richer as there are also associations that

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encompass the case of organisms that cannot live without each other any longer (mutualism), the most beautiful example of which is the eukaryotic cell, i.e., the association of a genome with a bacterium named mitochondrion. Parasitism and mutualism coexist within our biosphere and a game theory model has been proposed to summarise and analyse all the possible interactions (Renaud and de Meeûs, 1991). This simple model considers that host and parasite interact and that natural selection acts as an arbiter relative to the functions of utility of the game, i.e., the gains obtained by each of the two players. The game is based on two strategies that can be adopted by the host and the parasite and on the interactions between these strategies in terms of selective values for the two of them. This game, named «The Killer and The Diplomat» proposes a strategy of aggression and one of compromise. The question is: Parasitism: compromise or conflict? The game solutions can be defined in terms of Evolutionarily Stable Strategy (ESS, i.e., a strategy which cannot be invaded by any other strategy, thus conferring the adaptive optimum). The game solutions can thus be summarised as follows:

- There are two ESS (conflict or compromise) that are defined in function of the associated costs. Indeed, in the absence of costs related to resistance or virulence, there can be only one strategy (i.e., conflict, which implies the maximal virulence for the parasite and the maximal resistance for the host). However, costs associated with these two parameters (virulence and resistance) do exist and the examples in the literature are abundant (Burdon and Thrall, 2003; Sturm et al., 2011). For this reason, interactions tend to move towards situations of compromise in which the parasite minimizes its virulence and the host its resistance. This might seem trivial, but it had to be formally demonstrated. All the situations are thus feasible in nature in function of how the interactions will develop. Nevertheless, it is not possible to say, as often found in the literature, that a host/parasite association will always develop toward lesser virulence or lesser resistance;
- The utility functions of the game (the outcomes of the confrontations) then show that: (a) mutualism is just a form of parasitism in which the costs are positive, (b) commensalism (where the host accommodates a commensal that does not exploit the host) is a point of non-equilibrium that may sway towards mutualism or parasitism and so on, (c) parasitoids (i.e., very virulent parasites) are just organisms that have progressed towards the "conflict" configuration of the game.

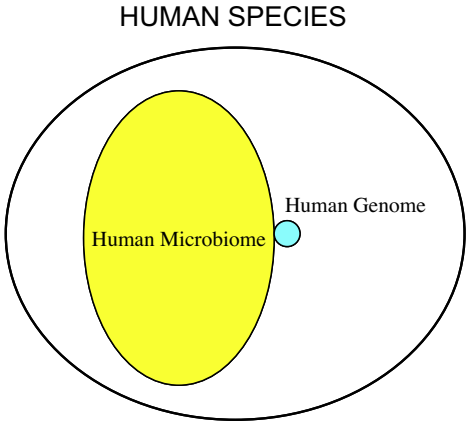

Fig. 1. The microbiome is the expression of the ecological conditions of the milieu (such as temperature, pH, content in hormones, lipids and proteins, UV exposure, absence of light, type of mucosa...), to which the involved microbial communities (which are defined as microbiota or flora) will react, individually and/or collectively, and which they can modify or maintain. It is currently thought that the micro-organisms living on or within a human being exceed of a factor of 10 the number of cells that constitute the human body. However, only 1% of them are currently referenced. The bacteria of the human body represent millions of genes in comparison to the 23,000 genes of the human genome.

Accordingly, the game solution is twofold and the evolution of a biotic interaction can move towards cooperation (i.e., mutualism) or aggression (i.e., parasitism). The cursor is thus variable.

The multitude of interactions that governs, at least partially, the evolution of the species of our planet concerns all (viral, bacterial, animal or vegetal) living organisms. The modalities of these interactions are many as our world is rich in life forms and an entire existence would not be enough to make the inventory of all of them. Hence, we will try to describe this richness by choosing to focus on the parasites and pathogens of the species that concerns us most: the human species.

1. Humans, their genome and the diversity of their symbionts

Humans, who are considered by many among us as the epitome of evolution, cannot live alone, that is without the symbionts that are associated with them. Generally, a eukaryotic organism cannot be considered as autonomous without the contribution of a multitude of organisms that are defined as its microbiome. A study published in 2007 showed that 90% of the cells in the human body are bacteria, fungi, protozoa (i.e., non-human cells). The current estimation of the human microbiome (i.e., the organisms that live on or inside *Homo sapiens*) exceeds of a factor of 10 the number of somatic and germinal cells of an individual (Turnbaugh et al., 2007). If we consider only the human intestine as an example, it contains on average 40,000 species of bacteria (Frank and Pace, 2008) and 9 million of unique bacterial genes [(Yang et al., 2009) – Fig. 1]. The immensity of this gene diversity can be better appreciated when compared to the 23,000 genes that compose the human genome. However, at most only 1% of the human microbiome has been characterised so far (Marcy et al., 2007). Therefore, we cannot any longer consider any organism as isolated, as we are all superorganisms the metabolic functions of which are the result of the expression of micro-organisms and human genes (Gill et al., 2006). One of the most illustrative examples is the gut microbiota, the densest microbial population in the human body. Kovatcheva-Datchary et al. (2013) describe this population as an organ itself, composed of 1000–1200 cell types (species) that encode 150-fold more genes (microbiome) than we have in our own genome. They report that it plays a fundamental role in human health, as it evolved specific functions that complement human metabolism and physiology. As example, intestinal bacteria take part in vitamin production, regulation of hormone synthesis, and maturation of the immune system.

Nevertheless, the interactions between all these symbiotic organisms are not always polite and mutually favourable; there are scroungers that can become pathogens. As example, Taur and Pamer (2013) explain that the modification of the intestinal microbiota and especially the loss of the microbial stability in humans can lead to the overgrowth or domination of pathogenic bacteria. Indeed, in the case of immune suppression, antibiotics as well as impairment of host immunity can cause the disruption of the microbiota giving rise to perturbations favouring intestinal domination by pathogenic species.

All the complexity of the relationships among living organisms resides in the type of interactions that they establish with their neighbours. Accordingly, resistance to antibiotics, which is a major public health issue, has been around since the dawn of time. This resistance appeared well before the use by humans of medicinal drugs. Indeed, a very recent study on sediments of the Canadian

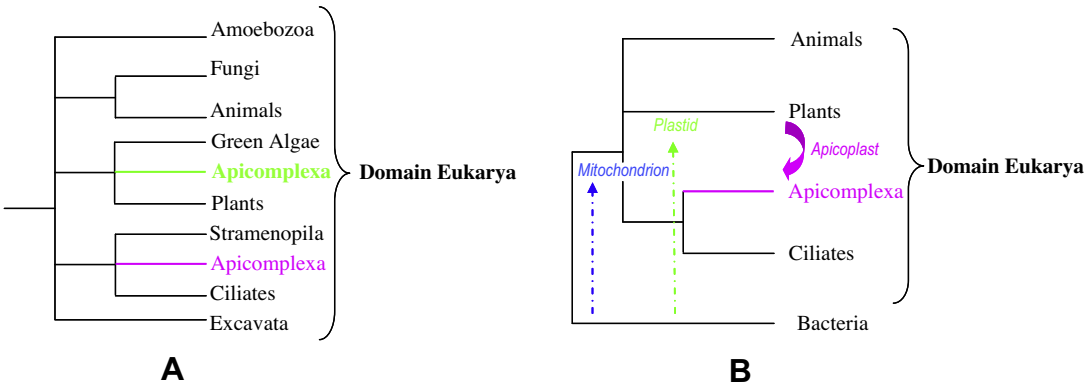

Fig. 2. The parasites that belong to the Apicomplexa group are the result of a double symbiosis. (A) Based on their morphological, biochemical and molecular (nucleus and mitochondria) characteristics, their phylogenetic position is together with Ciliates and Stramenopila (position in mauve). If the presence of the plastid (circular DNA of 35 kb) within their cytoplasm is taken into account, Apicomplexa should be classified between plants and algae (position in green). (B) Parsimonious hypothesis about the origin of Apicomplexa as derived from a double symbiotic acquisition of (i) a mitochondrion like all eukaryotes and of (ii) a plastid from green algae that indicates a lateral transfer which gave rise to the apicoplast. The ancestor of Apicomplexa was presumably a protozoan that ate algae. Modified from Roos et al. (1999).

permafrost has demonstrated the existence of ancient bacterial sequences that correspond to resistance genes which certainly allowed these bacteria to defend themselves against aggressors (D'Costa et al., 2011).

2. The multiple features of *Plasmodium*

The world of host/pathogen relationships is immense and its diversity can sometimes be overwhelming. This is well illustrated by the example of malaria which is the first parasitic disease in the world caused by unicellular eukaryotes. This disease, which still kills about one million children per year, is caused by blood parasites, protozoa of the genus *Plasmodium*. One might think that this is a very simple organism because it is unicellular.... Far from it. Let's see why.

The *Plasmodium* parasites belong to the Apicomplexa group like, for instance, the *Toxoplasma* species. If we analyse the phyletic position of these parasites, we see that they are very close to ciliates in the evolutionary tree (Fig. 2A). Indeed, these organisms have morphological, molecular, biochemical and pharmacological characteristics that bring them close to ciliates and dinoflagellates (Levine, 1988; Roos et al., 1999). Nevertheless, the discovery of a circular DNA of 35 kb incorporated in the cytoplasm of Apicomplexa has been a puzzle for a long time. Molecular sequencing of this element showed that it was much closer to the plastids found in algae than to a cyanobacterium or any other free prokaryote (Kohler et al., 1997). Thus, the presence of this plastid suggests that Apicomplexa should be rather grouped with plants. The most parsimonious hypothesis to explain this situation is the existence of a lateral transfer of an algal plastid that occurred in the ancestor of Apicomplexa. These organisms should thus be the result of a double symbiosis (Fig. 2B): (1) the acquisition of mitochondria like all eukaryotes and (2) the secondary integration of a plastid that led to a new symbiosis at the origin of the apicoplastid. In other words, the ancestor of the Apicomplexa should have been a eukaryotic protozoan that fed on algae and this «algaevory» allowed the integration of the plastid in the structure of the protozoan, giving birth to the Apicomplexa group. How did they become parasites remains a mystery; however, it must be acknowledged that this double symbiotic event has been a success because the group is much diversified. Just as an example, it has been possible to catalogue more than 180 species of the genus *Plasmodium* that parasite Reptiles, Birds and Mammals. But there is still more to come....

If humans have two separated sexes (female and male), this is not the case for *Plasmodium*. They are anyhow sexuated organisms, because the same genotype produces male and female gametes. They are hermaphrodites. Moreover, in the cycle of these parasites there are also phases of asexual mitotic reproduction. Thus, asexuality and sexuality are two assets of the biology of reproduction of these parasites. Indeed, two studies carried out in Kenya (Razakandrainibe et al., 2005) and Cameroun (Annan et al., 2007) showed that they can undergo self-fertilisation (i.e., here, union of gametes from the same individual, with the same genotype) and out-crossing (union of gametes originating from two different genotypes). Even better, it has been demonstrated that reproduction in this parasite is due for ¼ to self-fertilisation and for ¾ to out-crossing. These organisms have thus a policy of reproductive assurance to maintain their genotype by self-fertilisation and propose new combinations through out-crossing (Dornier et al., 2008). Furthermore, another work has demonstrated that these parasites can also adjust their sex ratio (i.e., differential production of female and male gametes) to optimise their chances of fertilisation, and thus their fitness (Paul et al., 2000).

These protozoa are «Machiavellian» because they present all possible reproductive options: sexuality, asexuality, self-fertilisation, out-crossing, sex ratio adjustment... Such large reproductive choice gives an enormous advantage to these parasites, and therefore it is easy to understand the difficulty humans have to face for putting in place effective means to fight them.

3. Human evolution and parasitism

What roles do pathogens play in the ecology and evolution of the living world? Terrific question. The time when only parasitologists were interested in parasites is well and truly over. Nowadays, the influence of parasitism on the evolution of living organisms is for some scientists a major research theme and for others an option that must obligatorily be taken into account given the fact that all living organisms are concerned by parasitism. If in the past, work was focused mainly on the direct effects of pathogens on the fecundity and survival of their hosts, the current research is highlighting their influence on host traits as different as behaviour, morphology and physiology, to mention only a few. And what about the human species?

During their evolution, humans have always lived in contact with rich and diversified communities of pathogens. For instance, although the habitat of the first hominids was limited to tropical savannahs, human migrations as well as the colonisation of more temperate regions have been accompanied by the exposure to new parasites (Armelagos et al., 1996). The structure and the size

of these human communities did also have a major influence on the dynamics and diversity of the parasite populations. Before the Neolithic, the social groups were mostly too modest in size to allow the maintenance of endemic diseases. An important change during the Neolithic was the organisation of sedentary societies around villages that grouped together many more individuals. An almost immediate consequence was the appearance and maintenance of many infectious diseases, such as measles, mumps, flu and smallpox. Always during the Neolithic, the proximity between humans and animals was increased by animal domestication, a phenomenon at the origin of a recrudescence of zoonoses (Polgar, 1964). Many years later, during their voyages, European explorers spread several contagious diseases (whooping cough, measles, smallpox...) that decimated many autochthonous populations (e.g., Amerindians), thousands and thousands of kilometers away from their country of origin.

Therefore it is unconceivable that the species *Homo sapiens*, who is nothing else than a particular animal, would not have been influenced during its evolution by parasitic constraints. It is worth noting that infectious disease mortality has remained virtually unchanged from Paleolithic times since the advent of antibiotics and vaccines, with life expectancy of only ~25 years (Casanova and Abel, 2005). In developed countries, it is easily forgotten that an infection nowadays can be easily cured by a simple antibiotic prescription would have been lethal only few decades ago. This apparently simple concept is heavy in consequences and gives an idea of the major role played by parasitic constraints in human history. Moreover, the phenomenon is far from being over as even nowadays parasites (in a broad sense) kill directly or indirectly millions of people each year. Like all living organisms, humans have been and remain a species subject to natural selection and pathogens are clearly part of these selective pressures. Consciously or not, humans have developed, like other species, multiple strategies to avoid the risks and/or the consequences of infections for themselves or their descendants. A review of the many articles published during the last 15 years in human evolutionary ecology leads to the conclusion that many aspects of human biology, particularly human life-history patterns, many behaviours and possibly also the cultural and religious diversity of human populations cannot be fully understood without taking into account the parasitic constraints (Thomas et al., 2012). We will now discuss a few examples of these parasitic influences.

Guégan et al. (2001) have, for instance, shown that fertility variations in human populations are at least in part indirectly imputable to the diversity of the pathogen communities. Fertility increases when the diversity of severe infectious diseases (such as malaria, yellow fever, dengue fever, cholera...) also increases. The elevated child mortality linked to diseases might thus be associated with compensatory responses that aim at producing more children. In addition to the description of a theoretically predictable adaptive response, this work also suggests that the global fight against parasitic diseases should paradoxically slow down human demography, because, on the long term, it would cause a reduction of fertility (by stopping the compensatory effect) in the countries that currently are the most exposed to diseases. Birth weight could also be influenced by parasitic pressures. Although health specialists often think that this variable is exclusively determined by the current environmental constraints, several evolutionists have proposed that part of the variation could be due to the influence of natural selection. Thomas and coll. (Thomas et al., 2004) have indeed shown that when the number of severe infectious diseases rises beyond a certain threshold, a concomitant increase of the average birth weight will be also observed. As big babies are usual more resistant to infections or tolerate better their consequences, this result is compatible with the hypothesis that humans (like any other mammal) maximise their selective value not only through the number of their descendants, but also by giving birth to babies with an optimal weight relative to the local environmental constraints.

When human beings do not die of old age, accidents or parasitic diseases, they die of somatic diseases (e.g., cancers, cardiovascular diseases, mental diseases). Although generally parasitic and somatic diseases are considered as opposite entities, an increasing number of works indicate that many diseases which are thought to be of "non-parasitic" origin have in reality an infectious cause (Cochran et al., 2000). These findings are of crucial importance because infections are often preventable (by vaccination, for instance) or treatable and thus the associated somatic diseases could also be easily avoided. Cancer is particularly interesting from this point of view. At the beginning of the 70s, about 1% of cancers were considered to be of parasitic or viral origin. Now, the World Health Organisation acknowledges that 20% of cancers are due to infectious agents, particularly RNA and DNA viruses and bacteria. For instance, hepatocellular carcinoma, stomach cancer and cervical cancer can be largely attributed to the hepatitis virus B and C, the bacterium *Helicobacter pylori* and to papillomaviruses, respectively. Some evolutionists, like (Ewald, 2009), think that within 2050 the great majority of cancers will be considered to have an infectious origin. What are the characteristics of oncogenic pathogens and how can they transform normal cells into cancer cells? Most of the pathogens that can cause cancers are pathogens with sexual transmission (intercourse, heavy petting) that leads to a relatively low transmission rate (in comparison to viruses, like the one of influenza); on the other hand, the infection is persistent and often cannot be eradicated. This feature is important for understanding why and how these viruses can lead to the cell deregulation which is at the origin of cancer. According to Ewald (2009), normal cells have put in place at least four barriers to avoid sinking into the unicellular selfishness of cancer cells: (i) they divide only when they receive the appropriate signal; (ii) when they accumulate too many genetic anomalies, they commit suicide (by apoptosis); (iii) they can divide only for a limited number of times (differently from cancer cells that have become immortal thanks to telomerase activation); and, finally, (iv) their adhesive properties do not allow them to detach and to migrate somewhere else, like cancer cells do during the phase of generalization of the disease (metastasis formation). Interestingly, although oncogenic pathogens are phylogenetically diversified, they all can sabotage these four barriers to favour their survival and transmission during the long years they must spend in their host and avoid the immune system. The problem of this sabotage is that it allows the accumulation of mutations without elimination (by apoptosis, for instance) of the cells that have been thus modified. The quantity of mutations will be more elevated when exposure to mutagens (tobacco, alcohol...) is more important. When mutations will touch systems that regulate the four anti-cancer barriers, cells will be no longer simply sabotaged or "manipulated" by a pathogen for its own replication strategy, they will then be transformed into cancer cells: at that point neither the host nor the pathogen will be able to control their anarchic divisions. There are many other proximate mechanisms through which pathogens can transform healthy cells into cancer cells, such as the induction of chromosome instability and of translocations, inflammation...[see (zur Hausen, 2009) for a review]. Indeed, the infectious origin of cancers is one of the hottest current research topics.

In what way the knowledge, or better the acknowledgment of the evolutionary interactions between humans and pathogens is important at this point in time? Humans are increasingly able to act on the parasites which threaten them, but often without worrying about the consequences that might result from disequilibria in the evolutionary dynamics that they maintain with these same pathogens. If intuitively it seems desirable to get rid of the parasitic

burdens, it must not be forgotten that these burdens constitute a major evolutionary force without which the world would be different.

In recent times, in some regions, the contacts with pathogens have strongly decreased due to important social changes. For instance, the generalization of sanitation, marsh draining and the changes in farming practices and lifestyle during the 19th and 20th centuries played a major role in the disappearance of malaria in West Europe. More generally, the changes in life hygiene and the generalization of immunization during the 20th century, as well as the growing use of antibiotics starting from the 50s, have led to an unparalleled reduction of the parasitic burden. This had important positive consequences due to the strong, deleterious effects of most parasites. For instance, the increase in life expectancy, traditionally attributed only to the progress of medicine, results essentially from the reduction of parasitic contacts, to which medicine has definitely contributed. It remains to be understood and this has started to be tackled, to which extent the parasite reduction has also contributed to other changes of this century, such as the increase of birth weight, the reduction of fertility, the increase of QI and so on.

The reduction in the contacts with parasites had negative consequences as well, because the host-parasite co-evolution generated complex interactions. For instance, intestinal parasites, such as helminths, have developed the capacity to regulate the type 2 immunity in order to increase their survival. On the other hand, the regulation of the immune system has also evolved in order to reinstate a normal expression in the presence of helminths. Thus, the abrupt destruction of all the intestinal worms favours an immune dysfunction because (in the case of helminths) a regulatory factor is suppressed, leading to an inappropriate (autoimmune) immune reaction. The recent, important emergence of different allergies, Crohn's disease, type 1 diabetes, asthma and many other disorders might also be explained in such way. The finding that the symptoms of Crohn's disease can disappear following the ingestion of the eggs of a parasite of another species (that hatch but do not develop) paves the way to many applications for the treatment of these diseases with non-pathogen parasites. Furthermore, the selection for strong immune responses in countries with elevated parasite pressures is associated with inflammatory responses that might favour the appearance of cancer later in life. This phenomenon, made evident by the recent lifespan lengthening, suggests that, unfortunately, cancer will be a future major health issue in developing countries. Understanding the modifications of the interactions between humans, their parasites and health in general is certainly a promising topic as shown by the current expansion of Darwinian medicine.

4. The human species: an ecosystem among others

Infectious (bacterial, viral, fungal and parasitic) diseases still have a devastating impact on human health. Twenty percent of mortality worldwide and 60% in Africa are due to infectious diseases, thus representing more than 17 million victims each year. Nevertheless, the causes of phenomena of recurrence, emergence and re-emergence of these diseases are too often not well known. This is due to mainly the complexity of the infectious systems in which a variety of parameters related to the vectors for vector-borne diseases, the environment, the hosts and the pathogens act and interact. Moreover, many factors can modify the epidemiological context and thus the dynamics and the spreading of an infectious disease. These modifications can be related to changes in the ecosystem, to the capacities of adaptation of the infectious agents, or to the exposure of individuals to agents that up to that moment were confined to a non-anthropised ecological niche. Among the thousand human pathogens that have been identified so far, more than half have a zoonotic origin (like, for instance, the Ebola virus, the virus of avian flu, some species of *Leishmania* responsible for leishmaniases, the borrelia species, the bacteria responsible for Lyme disease) and an important part of these pathogens are carried by arthropod vectors (the virus of dengue fever, the plasmodia, the different *Leishmania*...). Moreover, recent works still highlight the existence of new reservoirs and new vectors for diseases that have been known already for many decades, as well as the identification of new micro-organisms (Leroy et al., 2009; Ollomo et al., 2009; Prugnolle et al., 2010; Zou et al., 2010; Rougeron et al., 2011b; Schex et al., 2011; Senghor et al., 2011; Uhlemann et al., 2011; Vasilakis et al., 2011; Godreuil et al., 2012; Senghor et al., unpublished data). This suggests that many micro-organisms and many biological (vectors, reservoirs) and/or ecological (environment) niches remain to be discovered. We are thus still far from knowing all the infectious systems and particularly the extent and distribution of micro-organisms that are pathogen or not for humans.

An element that increases our difficulty at grasping the real extent of the distribution and genetic diversity of micro-organisms in the ecosystems is the focalisation of many research projects on pathogens. Indeed, the strains used for the studies on virulence, resistance, genomics, experimental evolution, population genetics, and so on, are in general clinical isolates (Rougeron et al., 2009, 2011a; Marsden et al., 2010; Downing et al., 2011; Eppinger et al., 2011; Muller et al., 2011; Snitkin et al., 2011). This approach axed mainly on human patients and thus on the micro-organism as an infectious agent is largely reductive because the role of a micro-organism as a human pathogen represents very often only a minimal portion of its life-history patterns. This has been termed 'iceberg bias' (Tibayrenc, 1999). This is the case, for instance, of *Mycobacterium bovis* or of the virus of mad cow disease (de la Rua-Domenech, 2006; Hlavsa et al., 2008; Imran and Mahmood, 2011a,b). Indeed, in many infectious systems, the human species is only an accidental host and very often an epidemiological impasse due to the medical treatments or because it is not a reservoir, or due to its rapid death. This is true for *Mycobacterium marinum*, a fish bacterium, Ebola virus or even *Leishmania infantum*, responsible for canine leishmaniasis. The human compartment can thus be seen as negligible for the majority of micro-organisms. Moreover, some viruses or parasites have been considered for a long time as confined only to humans, but have all the potential ecological niches that allowed them to survive in the system before emerging in humans been thoroughly elucidated? For instance, *Plasmodium falciparum* has recently been identified in gorillas (Prugnolle et al., 2010) and Ebola virus in bats (Leroy et al., 2009).

A micro-organism is identified as a pathogen when the host immune system cannot manage to control the infection and it causes physiological malfunctions and clinical symptoms. The immune response is heterogeneous in humans and can vary in function of the genotype of the infecting organism and/or of the physiological status of the host (Beck and Levander, 2000; Maciel et al., 2008; Ajzenberg et al., 2009). Moreover, epidemiological studies show that people are much more sensitive to infections when the affected population is naive of all contacts with the emerging organism (Alcais et al., 1997; Camargo et al., 1999; Bucheton et al., 2002). These elements show how the classification as pathogen for any given species of micro-organisms depends not only on the physiological status of the infected individual, but also on the immune status of the studied population.

As a general rule, most microbes exist and circulate as non-pathogens independently of the environment and both in vertebrate and invertebrate hosts. They can multiply and spread without systematically causing damage to the host that accommodate them. This is true also for humans. Indeed, although it is not very much studied due to the absence of medical interest, the

asymptomatic carriage seems to be the rule rather than the exception. From birth, humans live with a multitude of harmless or even beneficial micro-organisms. However, it is well known that these micro-organisms, these human symbionts, can become pathogens in people with a poor immune system, such as for instance individuals with AIDS. Conversely, many infectious agents that have been identified as human pathogens can circulate in humans without producing apparent clinical symptoms. Indeed, it has been clearly demonstrated that asymptomatic carriers (i.e., infected individuals without apparent clinical symptoms) represent the great majority of the human population for numerous infectious diseases. This assumption is true for many parasitic diseases (Chagas disease, leishmaniases, toxoplasmosis or malaria), but also for viral diseases (herpes or hepatitis) as well as for bacterial diseases (tuberculosis and staphylococcosis). This compartment is all the more important as it can play a role in the transmission of infectious diseases. This is the case of *Staphylococcus aureus* that circulates asymptomatically in more than 30% of the human population or for *Toxoplasma gondii*. For leishmaniases, this compartment has been known for about 10 years and the prevalence of asymptomatic carriers is higher than 30% in some studies (see for review Bañuls et al., 2011). These works clearly show that the number of asymptomatic carriers of the parasite *Leishmania* is without any doubt higher than the number of cases of symptomatic leishmaniasis. Similarly, for *Mycobacterium tuberculosis*, the agent of pulmonary tuberculosis, the majority of infections are asymptomatic (estimated at 1/3 of the world population) and remain latent and only some people will develop active tuberculosis following infection (Brodie and Schluger, 2005). This compartment represents thus an unlimited reservoir of pathogens and a major obstacle to the eradication of tuberculosis (see review Godreuil et al., 2007). Moreover, it should be noted that this bacterial population, although considered as dormant, seems to evolve and keeps acquiring mutations during the latency phase (Ford et al., 2011).

The human-centred approaches, which are mainly focused on micro-organisms as human pathogens, are at the origin of important biases in all research fields (population genetics, epidemiology and biology) concerning the interpretation of data and the understanding of the infectious systems. The direct consequence of this reductive approach is that we do not have access to all the biodiversity of the micro-organism under study. Yet, this biodiversity is crucial for identifying and understanding the processes of transmission, adaptation, evolution and co-evolution and also of virulence.

For population genetic studies, the knowledge of the global genetic diversity is a pre-requisite to determine the reproduction strategies and the population structure. For many micro-organisms, it is impossible or difficult to study experimentally the mode of reproduction, and only theoretical analyses based on the study of the genotype distributions and allele frequencies allow predicting the systems of reproduction and the evolution of populations. In this context, to validate predictions, the sample must be representative of the allelic diversity of the studied population. Therefore, sampling should be as exhaustive as possible by integrating all the compartments of the system (vectors, hosts reservoirs, environment, symptomatic and asymptomatic carriers). This is even more crucial because the systems of reproduction in micro-organisms can be complex and manifest specific features in function of the hosts they invade during the cycle. For instance, *T. gondii*, the agent of toxoplasmosis, uses a sexual reproduction system only in cats (definitive host) and an asexual system in all the other hosts (humans, sheep, birds, mice...). Similarly, *P. falciparum* moves from sexual recombination in its vector to asexual reproduction in its vertebrate host. This clearly shows that human-centred studies and sampling bias can generate important errors of interpretation concerning the biology of these micro-organisms. A typical example is represented by the *Leishmania* model (the agent responsible for leishmaniasis), in which past analyses generated debatable and incomplete hypotheses concerning the system of reproduction (for more details, see Rougeron et al., 2009, 2010, 2011a; Tibayrenc and Ayala, 2012). To study the population structure, non-representative samples of such populations and sub-populations are also an obstacle to robust analyses. Indeed, living organisms are generally organised in sub-populations and they are not distributed homogeneously in the environment. It is thus essential to take into consideration this sub-population structure because it influences the distribution of the genetic information and allows to avoid Wahlund effect (reduction of heterozygosity relative to the Hardy-Weinberg equilibrium due to subdivision of the population in several sub-populations that do not exchange gametes, or very little) and thus a misinterpretation of data (De Meeûs et al., 2006; Prugnolle and De Meeûs, 2008, 2010; Rougeron et al., 2009, 2010, 2011c).

The other research fields that are sensitive to the choice of microbial populations as study samples are the studies on the host-parasite (this term is used here in a broad sense) interactions, the biological mechanisms of virulence, resistance and pathogenicity, as well as on the associated genetic determinisms. As said before, in order to try to understand the polymorphisms that are associated with virulence and the clinical and resistance patterns that are common to most pathogens, these research works are generally focused on clinical strains. The difficulty in these studies is to distinguish between pathogen- and host-specific mechanisms. Indeed, the physiological state of the hosts and particularly of their immune system has an impact on symptom progression and on the clinical outcome of patients (Beck and Levander, 2000; Maciel et al., 2008). Therefore, it is difficult to target the genes and the regulatory mechanisms that are put in place by the micro-organisms to infect their hosts and, consequently, also to explain their pathogenic features. A line of research that has not yet been exploited enough in the field of infectious diseases is the comparison between pathogenic and non-pathogenic forms of a micro-organism, although it has been done for *S. aureus* for a long time. The comparison of the community and clinical strains or non infecting/infecting strains has brought important information on the mechanisms of virulence in these bacteria (Feng et al., 2008; Sotto et al., 2008; Otto, 2013). This type of study could also bring precious information for understanding the pathogenic potential of many other micro-organisms. For the model *Leishmania*, each clinical form of the disease is statistically associated with a specific *Leishmania* species (Bañuls et al., 2007; Bañuls et al., 2011). However, there is also an intra-specific clinical polymorphism and the role played by the parasite in it is not really known. So far, in both *in vivo* and *in vitro* models, only the strains from patients with more or less severe clinical forms have been studied (see review Bañuls et al., 2007). In these works, some rare associations were identified, but they could not be validated as general rules (see review Bañuls et al., 2007). The genomic, phenotypic and experimental (*in vivo* and *in vitro* models) comparison of strains of the same species from asymptomatic carriers and from symptomatic patients could bring fundamental information on the pathogenic polymorphisms of these parasites and on the involved biological and genetic mechanisms. This is particularly relevant as the first comparative molecular studies show a genetic differentiation between pathogenic and non-pathogenic forms of parasites (Bañuls et al., 2011, Hide et al., 2013), suggesting an intra-specific genetic determinism of the pathogenic capacity in *Leishmania*.

The resistance to anti-microbial compounds is also a field of research in which the human-centred approach can bias the interpretations and the decision-making in terms of control of infections. Indeed, the phenomena of resistance are generated by the drug selective pressure that is applied on the populations of

micro-organisms. This selective pressure exists of course in human medicine, but also in veterinary medicine with a comparable consumption of antibiotics in animals and humans. Some studies clearly show that the veterinary use of antibiotics leads to the emergence of resistance to anti-microbial drugs in humans as well (Smith et al., 2005; Shryock and Richwine, 2010). The first risk in human medicine is the transfer of resistant bacteria from animals to humans. It has been reported that the frequency of intestinal carriage of enterobacteria that are resistant to antibiotics used in veterinary medicine is significantly more important in farmers than in the urban population (Piddock, 1996; van den Bogaard et al., 1997; Kuhn et al., 2005; Smith et al., 2005). The second risk is the horizontal transfer of resistance genes from the bacteria hosted by the animal to pathogenic bacteria or human commensals. Indeed, the digestive tract of animals and humans is an ecosystem colonised by a microbial flora in which exchanges can occur as well as the dissemination of resistance genes within the bacterial populations that colonise it (Werner et al., 2000; Smith et al., 2005). These examples of resistance transfer between the animal world and humans show yet again the risk of limiting the studies to human pathogens and the necessity of trying to understand the populations of micro-organisms in their totality by integrating all the ecosystems in which they develop and interact.

All these elements emphasise that still nowadays we have access only to a minimal part of the population of micro-organisms we live and evolve with. Yet they have a considerable impact on the human populations in terms of demography and public health not only as symbionts, but also and particularly as pathogens. Indeed, infectious diseases have accompanied the human species all along its history and have contributed to shaping its evolution.

5. Parasites have shaped and still are shaping the human genome

During their evolution, all organisms have been and are challenged by parasites. Humans have not escaped this rule either, and if they managed to eliminate predators and competitors, this was not case for pathogens. Hence, the human genetic history is shaped by events that trace back the relations between humans and the pathogens that attacked and still attack them. We are not going to review here all the human genes that are marked by the contact with parasites (Barreiro and Quintana-Murci, 2010), we will consider just the case of malaria. Drepanocytosis or sickle-cell anaemia is a genetic disease that causes a heavy public health burden, particularly in West Africa. This red blood cell disease was identified a century ago. It is caused by a point mutation that modifies the structure of the beta chain of haemoglobin by a simple substitution of an amino acid in position 6 (Herrick, 1910). Very quickly, scientists became aware of the existence of a paradox, because this disease, which is very severe in homozygotes who carry the mutant allele, had an abnormally high frequency in Central Africa. Indeed, this disease is a genetic burden that should have never been maintained in a context of Darwinian selection. How to explain this? The proposed hypothesis is that heterozygotes who carry one normal and one mutant allele are more resistant to the parasitic attack by the malaria agents (i.e., *P. falciparum*) than homozygotes who carry two non-mutated alleles (Haldane, 1949). This has been defined as «The malaria hypothesis». An article published in 2010 by Piel et al. (2010) brings the formal demonstration of the selection of this genetic disease because of the resistance it confers to the parasitic attack by *P. falciparum.*

Although we can read in some newspaper articles that humans are not subjected to biotic selective pressures any longer, here we have the proof of the maintenance of a genetic burden (i.e. drepanocytosis) because of an infectious disease (i.e., malaria).

Moreover, this genetic system of mutant haemoglobin is not the only one to confer an advantage in the combat against malaria. Indeed, another major classical example in this field concerns the *Duffy* gene (*FyFy*) (Cutbush et al., 1950). Specifically, the absence of antigenic determinants of the Duffy blood group system might procure a protection against *Plasmodium vivax* to the majority of individuals in Intertropical Africa (Miller et al., 1976). Nevertheless, it has been suggested that *P. vivax* could use other pathways to enter red blood cells and that Duffy negativity might no longer be a barrier to infection and transmission (Ménard et al., 2010; Mendes et al., 2011).

There are many other genetic mechanisms of resistance to malaria in humans, such as Glucose-6-phosphate dehydrogenase, Glycophorines, Globins and Hepatoglobins, to mention only the processes involved in the resistance. The reader may refer to the excellent review (Kwiatkowski, 2005) to know the details of the different processes that have been selected during the co-evolution of humans and the agents responsible for malaria.

Pathogens have always been and still are a source of diseases and mortality for humans; thus they impose strong selective pressures that are reflected in the many genetic signatures which are engraved in our genome. The knowledge of these genetic fundaments represents a source of essential information for progressing in the fields of public health, clinical research and epidemiology. On the topic of the shaping of the genome by the selective forces of pathogens, the exhaustive work on immunology carried out by Barreiro and Quintana-Murci is remarkable (Barreiro and Quintana-Murci, 2010).

Very recently, a study has analysed and compared the environmental and pathogenic factors that are responsible for the selective pressures on the human genome (Fumagalli et al., 2011). The results show clearly that parasitic attacks are the major culprits of the molecular signatures of the human genome in the different populations. Briefly, the authors show that 103 genes are significantly correlated in frequency with parasitic attacks, whereas no gene could be correlated with climatic factors or the ecosystem-related life conditions. Just as an example that concerns a group of pathogens discussed in the first part of this review, the study reveals that 13 genes have been selected in our genome to fight against *Leishmania* infections.

Therefore, as demonstrated in this review human populations have co-evolved with a wide range of organisms, pathogenic or not. This co-evolution has clearly an effect on the human immunobiology, since our microbial partners participate in our immune system development and in predisposition/protection from immune-related diseases. In this context, the "hygiene hypothesis" has been proposed by Strachan in the 1980s (Strachan, 1989). This hypothesis states that the improvement of life conditions in industrialized countries, leading in a relative sterilization of the world, have resulted in the alteration of the symbiont community in humans and thus would favour the development of chronic inflammatory disorders (for review, see Sironi and Clerici, 2010).

From the dawn of Humanity, humans have been living with parasites that keep attacking them without respite; this process is not over, far from that, because humans in their demographic expansion will be more and more confronted with their «enemies»; the battle has started a long time ago and the war is far from being won.

6. Conclusion: living with pathogens in the 21st century

The world population – 7 billion currently – will reach 9–10 billion, or even more, at the end of this century. A major challenge for

all the societies and politicians who govern them will be to feed these populations, and another one will be to assure their cohabitation with pathogens that have been always there, from the dawn of life on Earth, and that belong, like us, to the big book of living things. Chikungunya, dengue fever, AIDS, tuberculosis, malaria are there to remind us of this. However, already during the Palaeozoic era, more than 300 million years ago and long before the emergence of the human species, insects were parasitized by viruses (Theze et al., 2011). The danger does not come from predators against which humans can defend themselves and eliminate them, but from parasites/pathogens to which the human species is just one ecosystem among many others, and with which humans have to live in an intimate interaction that can be sometimes pacific, sometimes aggressive or even mortal for them. Now, all the mass productions that humans will develop in the future to feed themselves will offer to these pathogens new opportunities for propagation.

The symbiotic interactions between pathogens and their hosts present many facets that range from aggression (parasites/pathogens), when one of the two partners takes advantage of the other, to cooperation or mutualism, when the interaction brings an advantage to both partners. "We were not born pathogens, we become" the pathogens would tell us if they could talk..., because they did not decide knowingly to harm us. The mitochondrial endosymbiosis that is at the origin of the eukaryotic cell is a proof of that. But, why do some pathogens become dangerous for humans or, in other words, virulent? This is one of the fundamental questions of the biology of the 21st century that must be answered to ensure the public as well as the animal and plant health. Indeed, health problems do not concern only humans, although they focalise legitimately all our attention.

Without getting caught up in a rigid Neo-Darwinism, it is indisputable that in this host/parasite interaction, the «best» ones will win in a time T and in a space S. Thus, pathogenicity (or virulence) is just a phenotype – i.e., a product of the genome – a variable that depends on the genetic organisation of the pathogen and of the hosts and that, in a given time and space (ecosystem), confers an advantage to some individuals who survive and reproduce better than others. Nevertheless, this pathogenicity/virulence has a downside, because a parasite that eliminates too quickly its host, as a consequence, will "commit suicide". We will discuss the case of the H5N1 virus that is the centre of many debates (Gauthier-Clerc, 2011). This virus is the product of a specific ecosystem that has been generated and is maintained by humans: in some region of Earth where the population density is particularly high, poultry farms have become true "biological reactors" to which humans add constantly new raw material (birds, the host). This has allowed the emergence and the selection of highly pathogenic viral variants the rapid diffusion of which is favoured by the low genetic diversity of the farmed animals as they have been selected on criteria of productivity. This virulence is thus a phenotypic trait that can be selected and therefore counter-select.

However, surprisingly, the exploitation of pathogen diversity and of their ecosystem in the combat against their aggressions remains very little developed. Indeed, so far, the majority of the medicinal compounds (penicillin, artemisin, paracetamol, morphine, quinine and so on, the list would be too long) have come from the biodiversity of the vegetal world (essentially fungi and plants) and from the painstaking screening of this diversity by scientists to isolate active principles. Yet, pathogens associate with or fight against other pathogens within a host – and humans are one of these hosts – and it has been demonstrated that these associations of villains can be exploited for our own good. Indeed, co-infections by multiple pathogens can reduce their virulence (Alizon and Lion, 2011). This is the case of malaria in Madagascar, where the presence of intestinal worms (Nematodes) in children seems to increase their protection against infection by *P. falciparum*, the agent of malaria (Brutus et al., 2007). Conversely, herpes virus infection increases the infection rate of the human immunodeficiency virus (HIV-1), and the cofactor role of sexually transmissible bacterial infections in HIV transmission is now well established (Van de Perre et al., 2008). Thus, like all the other wild animals, parasites fight or help each other. Competition and cooperation, two laws of ecology and evolution, should be better exploited to understand and control infectious diseases.

But humans seem to have only one idea in their mind, to which they consecrate all their energy: the eradication of pathogens. However, at the risk of shocking, a good pathogen is certainly not a dead pathogen. If some of them were to disappear, others would become free to increase their virulence. Otherwise, all pathogens should be eradicated, which is obviously impossible. After the end of the Second World War, major medical and technological advances have nurtured huge hopes concerning the combat against infections. We must admit that, perhaps with the exception of smallpox, no infectious disease has really disappeared from Earth, quite the opposite.

In 1988, the international initiative for the global eradication of poliomyelitis was launched; however, in 2010, although poliomyelitis is now endemic only in four countries, 23 previously disease-free states were re-infected due to importation of the virus. Daily epidemiological reports are in charge of reminding us about the increasingly worrying progression of resistant pathogens: resistant *Escherichia coli*, resistant*M. tuberculosis*, resistant*S. aureus*, resistant*P. falciparum* and so on. These infectious attacks are scaring and the media do not miss the opportunity to play scaremongers. However, humans are not blameless: if virulence and resistance are two phenotypic characteristics of pathogens, it must be acknowledged that far too often they are favoured by the systematic use of antibiotics (Hawkey and Jones, 2009) or of other anti-pathogen compounds. But doctors need to treat the diseased hosts, and thus to use them. This is a spiral, the happy ending of which is far from obvious.

Tomorrow's combat requires other means. We are not any longer at the stage of the elimination of the infective process – objective which cannot be attained – but in that of its control and management. Biological diversity is the natural wealth of Earth, pathogens are part of this asset, and it is our duty to understand the ecological and evolutionary modalities of their interactions with their hosts to get a profit out of them. We must also understand the interactions between pathogens and exploit to our advantage the battles they sometimes engage with other parasites to favour the less virulent ones. This approach must now be applied globally and not at the individual's scale, like in the past. Our mentality must evolve and only the association/cooperation of doctors, evolutionary biologists, chemists, just to mention them, will allow us to progress. In the future battle against parasites, the phase concerning the treatment of infected patients is too reductive and comes too late: instead we must strive to put in place a common, global management of the risk and of the infectious attack. The future of human populations depends on this. But we must not forget that the animal and vegetal worlds are similarly concerned: the emergence of a pathogen which is virulent for rice, for instance, could indirectly cause as many deaths as the flu due to the risks of famine. Without an equilibrium built at the planetary scale, the fall will be inevitable.

7. Declaration of interest

Authors have no declarations of interest to report.

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