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Highlight

Perspectives of a most pestilent past☆

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> Then I saw when the Lamb broke one of the seven seals, and I heard one of the four living creatures saying as with a voice of thunder, "Come." I looked, and behold, a white horse, and he who sat on it had a bow; and a crown was given to him, and he went out conquering and to conquer.
>
> (Revelation 6:1-2)

So finally it has come to this. You saw it happening around you, in your community, then in your family, and now it is happening to you. You threw up more in the past week than ever before in your life, your lymph nodes have been swollen and sore for days, you have been delirious from the fever, rashes marring your skin. Alas, it is the pre-antibiotic era, or perhaps it is the post-antibiotic era – the powerful tools of the past lost to now ubiquitous resistances. Either way, medicine cannot provide you with a solution for your troubles, neatly tucked away inside a pill. Now it is a fight between you and the disease, and this uphill battle is one that you are not winning. The tips of your fingers have already blackened, hope would be out of place. In a lucid moment you recall a memory from your childhood, a memory of your beloved pet: how at the very end it curled up, still alive, but clearly aware of its looming death, and waiting for it calmly. It feels so strange right now –not even scary– but downright bizarre, to be in its place. Exhausted, you close your eyes, and take one last breath as your physiology begins to fail.

You have no mind, but if you did, it would be nothing like that of the large, unwieldy, multicellular organisms in which you can thrive. Would you even think of yourself as a unitary entity? It's difficult to tell when so much of you resides in identical copies that share no communication. Perhaps your identity would be that of your whole breed: *Yersinia pestis*. If you did have a mind, perhaps the only things it would have in common with other minds would be the drive to continue existing. Doubtlessly, you would fear leucocytes, the natural predator that could spell your doom; though you would have high hopes of eventually overwhelming it. Otherwise, notions such as status would be of no significance to you. This is a shame; after all, millions upon millions have died in your wake. Perhaps that would be something to be proud of. Perhaps you would still remember being catapulted by the Tartars, aboard a corpse, over the city walls as an act of biological warfare during the siege of Caffa, on the Crimean peninsula [1]. And you might recall hiding aboard Genoese trading ships that would eventually lead you all the way to Sicily, and to the most prosperous time of your past [2,3].

Chances are that you would be very fond of fleas, who so conveniently ferried you around, across great distances, to places where you can prosper. However, one of the greatest differences between your mind and others', would likely be your lack of thought dedicated to something as frivolous as finding someone to mate with. Your biology would ensure that your obsession with genetic optimization –and yes, you would no doubt have an obsession for genetic optimization– would express itself in other ways. Not mixing your genome with a similar one to shape new permutations for a phenotype tailored to your circumstances means a slower evolution from one generation to the next. You would not be concerned though, after all one of your generations takes hours, not years. Yet one card up your sleeve strikes you as a blessing: the plasmid. Readily taken up, much like learning a new skill, though with none of the effort and all of the efficacy. Suddenly you are able to withstand an antibiotic, or gain access to a tissue that was once beyond your ability to infect, or break down a molecule that you previously regarded as indigestible. The plasmid would doubtlessly be one of the things you would be most grateful for in the whole wide world.

You are a researcher, though sometimes you wonder whether you shouldn't think of yourself as a historian, or perhaps a detective. Granted, your tools have stayed the pipette and the thermocycler, but your passion is the history locked away in the genome of strains that once depopulated continents. You follow the faint trail of a few scattered nucleic acids from anonymous mass graves, meticulously skimming through the pages of 14th century chronicles or letters, and attempting to sieve facts out of *The Decameron*[1].

Making sense of it all is no trivial task, especially considering that at the time, the Black Death was attributed to the conjunction of Saturn, Jupiter, and Mars in the House of Aquarius, combined with the lunar eclipse two days prior. That much was detailed in a report to the king, authored by the Faculty of Medicine of the University of Paris. Remedies entailed the drinking of mixtures

☆ Article highlight based on "Bioluminescent tracing of a *Y. pestis* pCD1^{+}-mutant and *Yersinia pseudotuberculosis* in subcutaneously infected mice" by Yazhou Zhou *et al.* [20].

[1] A collection of tales by 14th century author Giovanni Boccaccio set in the outskirts of Florence during the Black Death.

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containing ground, roasted shells of freshly laid eggs, chives, and ale[2], but also collective flagellation and the brutal massacre of Jews by the ten thousands, wiping out hundreds of communities[3].

Luckily, you stand on the shoulders of giants, such as Alexander Yersin, who in 1894 identified the gram-negative coccobacillus later named *Y. pestis* [4,5], spotted in China. It was caught *in flagranti* as the causative agent of the third plague pandemic in the mid-1800s, assisted by congested fleas and infected rats, making it officially a zoonosis.

Given the similarity of the symptoms, responsibility for the two previous pandemics was attributed to the same bacterium, a conclusion that became strongly contested a century later. In the minds of some, the standard model formed by the trinity of flea, rat, and bacterium did not match the lethality, nor the speed of propagation [6,7], especially compared to the third pandemic [8]. Other possible culprits were evoked, among them the agents of anthrax, typhus, tuberculosis and hemorrhagic fever [2,9].

In 1998 and 2000, Drancourt and Raoult identified *Y. pestis* in the dental pulp of plague victims from the 14th, 16th, and 18th centuries [10], and so proudly concluded "We believe that we can end the controversy: Medieval Black Death was plague"- probably the best way *not* to end any controversy. Many contested that the Black Death spread way too fast to have been a zoonosis such as the bubonic plague and thus that the credit should go to something probably extinct and maybe ancestral to *Y. pestis* [11,12].

With the entry of real-time quantitative PCR (RT-qPCR) and the trivialisation of sequencing, the molecular legacy of *Y. pestis* finally met the requirements for a definitive conviction [9,13,14]. In only one decade, the field of aDNA (for "ancient DNA") had progressed from a few hundred base pair PCR products on an agarose gel to the high-throughput sequencing of the pathogens' entire genome. In 2011, a big collaboration around Johannes Krause published a draft genome of *Y. pestis* [15]. Three years later, Wagner et al. provided formal proof for the first pandemic, or Justinian plague, also having been caused by a now extinct *Y. pestis* variant [16].

The debate shifted from "was it *Y. pestis*?" to "sure, but which one?", and much effort went, and still is going, into figuring out which branch of the bacterium's family tree committed which series of outbreaks, and when. Identifying these genetic signatures may unravel the pathogen's migration routes, and could also explain differences in virulence, and overall behavior [9].

In 2016 Krause and colleagues refined the deciphering of the whereabouts of the bacterium at a large scale, both in space and time, from Spain to Russia, and from 1300 AD to the modern days [3]. They came to a series of major conclusions, including that the low genetic diversity of *Y. pestis* during the Black Death indeed supported the scenario of a single entry into Europe. However, the third pandemic actually derived from the Black Death hotspot in Europe *via* a wave of plague travelling back towards Asia, where it brought about the source population of contemporary plague epidemics [3,17].

The ink has barely dried on the latest addition to the continuously rewritten story of the White Rider. In mid-January 2018, Schmid et al. published a study in which they model the three possible transmission models: rat-borne, human ectoparasite, and pneumonic plague, and attempt to fit them to the mortality data recorded from nine outbreaks in different European cities during the Second Pandemic. The authors conclude that human ectoparasites appear to have been the dominant transmission mode for this pandemic, as the model fits best the data of seven cities [18]. Thereby, it would seem that we owe *Rattus rattus* a sincere apology, as the commensal rat was commonly held responsible for the spread of the disease, while body lice and human fleas, jumping directly from person to person, were the main culprits. Doubts had already been issued concerning the preponderant role of the rats by several researchers for various reasons, including their absence from archeological excavations [19], climatic incompatibilities, and a surprising lack of accounts of rats travelling hundreds of kilometers daily. This would also fit Krause's conjecture that the "the perceived increased virulence of the disease during the Black Death may not have been due to bacterial phenotype, but factors other than microbial genetics, such as environment, vector dynamics and host susceptibility" [15]. Yet, unambiguous experimental evidence of transmission from human lice and fleas to humans has yet to be provided [18].

By all means, in an overall context of deducing genealogy from nucleic acid sequence, Xiaoyi Wang and Ruifu Yang put into action the rather decent idea to verify the assumptions directly *via* the pathogen's performance in a living host in the highlighted article [20]. Their study demonstrates that *Y. pestis'* lymphoid tissue tropism is a common denominator with its ancestor, *Yersinia pseudotuberculosis* and that this is *a priori* linked to the pCD1 plasmid. More live studies hold certainly some potential to fill in the gaps of coherence between the contemporary and medieval plague dynamics.

You raise your buzzing head from your journey from the crumbling pages of the past, rubbing your aching temples. Maybe you open a few windows, the ones in the wall and a few more on your screen. Slowly, various news and facts trickle into your frame of reference.

You realize that plague is not a mere specter of the past, a medieval museum piece associated with spine-chilling figures draped in dark, ankle-length overcoats and wearing bird-like beak masks; those filled with flowers and spices, in order to cancel out the bad smells, thought to be the infection's vector according to the miasma theory. Plague still thrives and prospers nowadays, with Africa as its principal feeding ground, and this to an extent to be considered a re-emerging infectious disease [9,20]. Since the start of the political crisis in 2009 that severely crippled the country's healthcare system, Madagascar is currently the most severely affected country. According to the WHO, 2348 plague cases and 202 deaths were reported for the period of August to November 2017, the latest and heaviest outbreak. Moreover, most of the recent cases were due to pneumonic plague, the type that is airborne, highly infectious, and can lead to death in about a day. Needless to say, the replacement of destroyed clinics by traditional healers, who solicit deceased ancestors for help through mirrors, is problematic. A strong tradition of unearthing the corpses of family members on a regular basis during a ceremony of the "turning of the bones", does not exactly contribute to improve the situation either[4,5] [21].

You also notice that despite your phone's continued insistence that you are definitely in the 21st century, somewhere in the meanders of the Internet, some of your fellow humans keep zealously discussing the exact astrological constellations that triggered the Bubonic Plague. With fancy circular charts and exotic vocabulary, they kindly inform you that "Astrology can still be used for prediction and diagnosis with the same accuracy as in the time of King Philip. […] Today we have effective treatments that make it no more serious than a bad case of flu. This doesn't negate the fact of the accuracy of astrology and the validity of using it to warn of

[2] http://spartacus-educational.com/EXnormans10.html.
[3] www.historyguide.org/ancient/lecture29b.html.
[4] www.washingtonpost.com/news/in-sight/wp/2016/03/09/the-plague-alive-and-well-in-madagascar/?utm_term=.24ba2bfe4e80.
[5] www.who.int/csr/don/27-november-2017-plague-madagascar/en/.

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