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Humans, Evolutionary and Ecologic Forces Shaped the Phylogeography of Recently Emerged Diseases

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Abstract

Many infectious diseases have emerged and circulated around the world with the development of human civilizations and global commerce. Anthrax, plague and tularemia are three such zoonotic diseases that have been intensely studied through genome characterization and phylogeographic analyses. A few highly fit genotypes within each of the causative species represent the vast majority of observed disease cases. Mutational and selective forces working together create highly adapted pathogens, but this has to be coupled with ecological opportunities for global expansion. This Review describes the distributions of the bacteria that cause anthrax, plague and tularemia and investigates the forces that created a clonal structure in both these species, and specific groups within these species.

Background

Even diseases that were once thought to be quite ancient appear, in fact, to have recently emerged, shaped by evolutionary and ecologic forces in the last ten thousand years. The evidence for their recent emergence invariably comes from molecular genetic analysis of the causative pathogens, which has been greatly aided by the availability of complete genomes sequences. Pathogen genomes are not simple entities but rather can be thought of as a composite of many different types of loci that evolve at very dissimilar rates and are subject to greatly differing selective forces[1]. As a result, observed mutation rates within a single organism can differ by at least six orders of magnitude. Some short tandemly-repeated sequences may mutate at 10^{-3} events per generation[2, 3] in a genomic background in which the average nucleotide rate is only about 10^{-10} changes per generation[4, 5]. Because bacterial populations can be very large and represent a large number of generations, mutations, horizontal transfer, and recombination easily occur at rates fast enough (10^{-3} to 10^{-10} per nucleotide per cell division) to generate considerable genetic variation, perhaps even in minor subpopulations.

Selection may enhance locus diversity, suppress it, maintain it, or, in theory, have no effect, which would be considered neutral variation. Mutations almost always become fixed in populations at slower rates than the rates at which they are generated. The fixation or population substitution rate is greatly influenced by selection, pushing molecular evolution towards neutral genetic variation whenever possible. It is true that most variation will have a negative impact and be removed from the population by selection. However, some will remain that is neutral, slightly disadvantageous, or of little advantage in the pathogen's current niche. This genetic variation provides the potential to be highly adaptive in the future given the right opportunity. Thus, pathogen populations contain many individual genotypes possessing genetic adaptations that are possibly important for global expansion into novel niches. For clonal organisms, the lack of recombination means that single mutational changes can influence the entire genome,

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because if that locus is under selection, the rest of the loci will "hitchhike" along with the selected loci. Currently, molecular variation in all its forms can be precisely measured and modeled using ecological and evolutionary theory to understand past population dynamics of pathogens on multiple spatial scales, local through global.

Genetic diversity within a given pathogen species is rarely evenly distributed across its geographic range. This is because the most-fit genotypes are frequently the most geographically distributed despite being among the most recently derived lineages. The success of a genotype (increase in frequency) can be due to genetic-based adaptive advantages, or merely fortuitous stochastic events. For example, dispersal of recently emerged pathogens may be explosively accelerated by the presence of immunologically naïve and susceptible host species. In contrast, an adaptive radiation may be associated with host shifting facilitated by adaptive fortuitous mutational events or lateral transfer of novel adaptive genes to the pathogen. By definition a highly fit genotype is one that is able to reproduce more successfully than other genotypes. It is evident that highly fit clones become ecologically established in many global locations that contain very different biotic and abiotic environmental factors. As a result, environmental factors combine with dispersal abilities of each pathogen and time dependent evolutionary rates to determine the level of local and overall geographic differentiation. In recently emerged pathogens, phylogenetic patterns at global, regional and local scales show evidence of these forces if the appropriate genomic loci are examined.

Many different pathogenic bacteria are examples of highly fit clonal expansions, and generalization across species is possible to a certain extent. Three of the most highly studied bacterial examples are *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), and *Francisella tularensis* (tularemia). These zoonotic species are both continuing public health threats and the three most dreaded bacterial biological weapons agents. As with all zoonotic agents, these pathogens evolved, dispersed, and became ecologically established in animal populations in a manner that can be inferred from their present phylogeographic patterns. All three of these bacteria are relatively recently emerged pathogens with even more recently emerged highly fit clonal genotypes nested inside less fit lineages. In this review, we apply what we have learned from phylogeographic studies of *B. anthracis*, *Y. pestis*, and *F. tularensis* to understand the past transmission and phylogeographic patterns of bacterial diseases and how these events are shaped by pathogen ecology and humans. Historical transmission patterns reconstructed from analysis of the phylogeography of these three bacteria will lead to process insights enabling us to better predict the inevitable emergence of novel pathogens.

Bacillus anthracis

Although the geographic origin of anthrax has not been firmly established, it is clear that *B. anthracis* is derived from a *B. cereus* ancestor. This ancestor obtained two virulence plasmids and underwent a pleiotropic chromosomal mutation in a *plcR* transcriptional regulator gene[6]. The combination of these three evolutionary events in the appropriate genetic background made *B. anthracis* into a catastrophic animal disease that can be easily distinguished by its distinct pathology, ecology, and evolution from disease caused by *B. cereus* 6.

Ecologic and evolutionary patterns in *B. anthracis* are highly dependent upon its ability to form spores. The disease manifestation component of the transmission cycle appears to be strictly dependent on a mammalian host, and is coupled with an environmental reservoir component in which spores persist in soil in a quiescent state for a number of years. There may be cellular replication in the soil but this is controversial and not universally accepted[7]. Importantly, the stable spore stage allows long-range dispersal of the pathogen while "freezing", or at the very least greatly slowing, its evolutionary progression. Anthrax is an acute disease, killing most hosts within days or weeks, thereby greatly limiting dispersal of the pathogen via the infected

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live host. Rather, long distance transmission primarily occurs once the host is dead via human mediated transport of contaminated animal products such as meat, hides, hair, and bones.

B. anthracis isolates from all parts of the globe can be highly similar in their genomic sequences[8], which can make subtyping very difficult[9, 10]. However, once phylogenetically informative characters (*e.g.*, single nucleotide polymorphisms, or SNPs) were identified, they proved to be very stable with almost no homoplasy (*i.e.*, phylogenetic inconsistencies)[11]. The very low homoplasy level is evidence of a very recently emerged pathogen but also of a highly clonal propagation system. Among multiple whole genome sequences there is no evidence of recombination among lineages or with other species[8]. Reconstructing the global transmission patterns was achieved[12] using rare, but highly stable, phylogenetic SNP characters discovered by comparing multiple whole genome sequences[1].

There are three deeply rooted lineages within *B. anthracis* (groups A, B & C; Fig. 1), each with a different global distribution and importance to current disease incidence [8, 12,9, 10]. The dominant clonal lineage is group A (~90% of all known *B. anthracis* strains), which underwent a recent radiation (Fig. 1) and is now globally distributed[12]. Subclades within the A lineage are often locally successful but also may be widely distributed. For example, the infamous Ames clade (~1%) is locally established in south Texas but is also well represented in China[13]. The Vollum clade (~5%) also is widely distributed, as it has been commonly found in commodities worldwide, but its source population is uncertain. In strain collections, the most dominant A-subclades are western North American (WNA) and the trans Eurasian (TEA), which combined represent over 37% of all known *B. anthracis* strains[12]. Their genetic relationship is very close, with fewer than 100 SNPs separating these two groups[14]. The wide geographic distributions, prevalence, and short evolutionary separation among these A subclades suggest that they are highly fit clones that could owe their great success to either deterministic adaptive changes in their genomes, or stochastic forces. Although adaptive differences cannot be ruled out, obvious genomic and phenotypic differences among the A, B, and C lineages have not been identified. In contrast, molecular clock estimates associate the timing of the A radiation with major human activities, such as the domestication of animals, suggesting that the stochastic process of human mediated dispersal may explain the great success of this group[12]; sometimes chance is as important as genetic adaptation to the success of particular bacterial types. Together the B and C lineages account for just a small proportion of globally observed isolates. The B lineage is divided into two main subclades (B1, ~6%; B2, ~2%) found primarily in southern Africa [15] and Europe[16], respectively, where they appear to be ecologically established. Because spores are still frequently dispersed in animal-derived commodities, identifying atypical outbreaks due to commerce is critical so these events can be distinguished from the overall ecologically established population structure. For example, B2 outbreaks are common in Europe[16, 17] arguing that this subclade is ecologically established in this region. In contrast, a single B2 anthrax outbreak near San Jose, CA (USA) in 2001 may not be indicative of a well-established or widespread population and possibly reflects dispersal by human activity because other B2 isolates have not been found in this region. The most basal lineage, C, is very rare (~0.2% of known isolates) and has only been observed in North America where sampling has been very intense[12].

Yersinia pestis

The phylogeography of *Yersinia pestis* has also been shaped by its ecology and human-mediated dispersal. Plague is one of the most notorious diseases in human history due to three major pandemics during which millions of people were killed (see text box) [18]. Despite its impacts on human populations, plague is primarily a disease of rodents and their fleas[19]. Similar to *B. anthracis*, *Y. pestis* is a recently emerged clone of a closely-related species, *Y. pseudotuberculosis* 20. Age estimates based upon molecular clocks suggest that *Y. pestis* split

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from *Y. pseudotuberculosis* within the last 9,000-40,000 years [21]. Thus, within a relatively short period of time this species made a drastic niche shift from an enteric pathogen to an obligate blood-borne pathogen that continuously cycles between rodent hosts and flea vectors [19]. Genomic comparisons [22, 23] suggest this shift was facilitated by the acquisition of several genomic components not found in *Y. pseudotuberculosis*, including two additional virulence plasmids; one of these plasmids appears to have a recent common ancestry with a plasmid in *Salmonella enterica* serovar Typhi [24]. With the exception of these two plasmids and a small number of other acquisitions, the rest of the *Y. pestis* genome has been under decay and is greatly reduced compared to *Y. pseudotuberculosis*22, although what remains is quite similar probably because there has been little time for mutations to occur.

Y. pestis is very genetically monomorphic and, like *B. anthracis* and *F. tularensis*, the limited genetic diversity within this species is not evenly distributed across its global range. There are just three major branches in its phylogenetic tree and eight major molecular groups (Fig. 2). More molecular groups will no doubt be identified and defined once additional isolates from Central Asia are analyzed as this is likely where *Y. pestis* arose [25]. There are specific host associations in plague foci in Central Asia [26, 27] and this local differentiation and adaptation to different hosts and vector species over relatively long periods of time appears to be the main driver of genetic diversity within this species [27] as most of the diverse types are still limited to this region (Fig. 2). Indeed, only two of the eight major groups are found outside of Eurasia: the 1.ANT group is found in central Africa and the highly successful 1.ORI group is found on all continents except Australia and Antarctica (Fig. 2). Despite its global distribution, the 1.ORI group is very monomorphic due to a recent genetic bottleneck. This group was responsible for the 3rd pandemic in the 1800s and 1900s, during which it was introduced to Africa, Australia, North America, and South America[18]. Limited, but compelling, molecular evidence also suggests that the 1.ORI group was involved in the first two human pandemics[28, 29]. Although the orientalis biovar correlates well with the 1.ORI group, the other two biovars, mediaevalis and antigua, are not single monophyletic groups [25]. As such, *Y. pestis* biovars provide little help in understanding phylogeography patterns.

Y. pestis is highly monomorphic from a nucleotide perspective [25] and its population structure (Fig. 2) is consistent with a clonally propagating pathogen. Genomic rearrangements, mediated by insertion elements, do appear to be quite common [25, 30] and can be identified even among closely related strains [31]. However, it appears to be a strictly clonal pathogen as there is little evidence for genetic recombination among strains, or with other species [25]. Exceptions to the latter are the acquisition by *Y. pestis* of novel plasmids in addition to the three common plasmids [32], which has been reported by several authors [33-36]. Most troubling are two novel plasmids, isolated from two separate *Y. pestis* isolates in Madagascar, that contain multiple antibiotic-resistance genes [35, 36].

Y. pestis occurs in two distinct ecologic situations depending on the type of rodent species involved. The first situation, which is found in most of the ancient foci in Central Asia but also in North America foci and elsewhere, involves *Y. pestis* cycling within sylvatic, native rodent species that typically are burrow-dwelling or create large nests, which may be important for flea survival. The second situation is found in parts of Asia and Africa, especially in Madagascar, and is the situation associated with most human plague cases today. It involves primarily non-native commensal rodents (such as *Rattus* spp.) in large populations. These types of rodents and their associated fleas also are important for the dispersal and establishment of *Y. pestis* in new areas. This likely reflects the presence of commensal rodents, and thereby their fleas, on ships and other forms of transport. Indeed, *Rattus* spp. were the most important plague hosts during the third pandemic and have been implicated as important hosts during the first two pandemics.

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Probably because *Y. pestis* has the ability to infect a wide variety of rodent species, once introduced to a new region plague can transfer into and become endemic in native rodent species without the further involvement of non-native rodents, as is the case in North America where non-native rodents are no longer involved in plague ecology. If their numbers and geographic distributions are sufficiently large, *Y. pestis* can be maintained solely by non-native rodents. This is the current situation in Madagascar, where *Rattus* spp. greatly outnumber native rodents, none of which are involved in plague ecology. Conversely, if *Y. pestis* is introduced to a new area via non-native commensal rodents but these species are not widespread and there are no native rodents available to support plague, the disease apparently cannot become established. When *Y. pestis* was introduced in Australia during the third pandemic it briefly caused plague outbreaks among *Rattus* spp. and humans. But with no native rodents in Australia, coupled with improved hygiene conditions that reduced the number of non-native rodents, plague disappeared from this continent. Thus, the current global distribution of the 1.ORI group of *Y. pestis* is the result of both human-mediated dispersal and the specific ecologic situation in the invaded regions.

Francisella tularensis

F. tularensis is typically separated into four subspecies that directly correspond to robust molecular phylogenetic groups (Fig. 3), each of which have distinct geographic distributions and disease manifestations[37]. Two subspecies, *F. tularensis* subsp. *novicida* and *F. tularensis* subsp. *mediasiatica*, are rarely observed and at best opportunistic pathogens; they occur primarily in North America and Central Asia, respectively. Most human tularemia cases are caused either by *F. tularensis* subsp. *tularensis* (Type A), which is generally considered to be the most pathogenic subspecies, or *F. tularensis* subsp. *holarctica* (Type B).

F. tularensis subsp. *tularensis* is found in North America and recent genetic and genomic analyses have identified two distinct populations within this subspecies, A.I and A.II, that are nearly as genetically distinct as the other subspecies[38, 39, 40] Indeed, a recent North American epidemiological analysis suggested the A.II group may be less virulent than even *F. tularensis* subsp. *holarctica*[41]. The A.I and A.II groups have distinct geographic distributions, with the A.II subclade found in the western United States and the A.I subclade primarily found in the eastern US and occasionally California (Fig. 3). These distributions are also highly correlated with specific host and vector species [40]. However, it is not clear if these pathogen groups are specifically adapted for transmission by these particular vectors and hosts or if they just share similar geographic distributions and, hence, are just fortuitously associated. Future research needs to move past descriptive science to experimental tests of ecologic differences among the *F. tularensis* subspecies and groups. This should lead to an initial description of the overall transmission cycles of this pathogen, and may provide insights on ecologic differences that explain the distinct geographic distributions of the subspecies and groups. *F. tularensis* subsp. *tularensis* has been identified from the Old World[42] but this possibly represents a laboratory escape following human transport, as genotyping[39] and genome sequencing[43] have established its close identity to the common lab strain SchuS4 (Type A.I).

F. tularensis subsp. *holarctica* is found throughout much of the Northern Hemisphere and contains little genetic diversity[37, 39]. This pattern is consistent with a very recent emergence, although there is enough phylogeographic differentiation to suggest the origin of its emergence and global dispersal. The most ancestral lineage is the unusual "*japonica*" group, which has only been observed in Japan[44]. Likewise, the second most ancestral lineage has only been observed in California, USA[40]. Using whole genome analysis, Vogler and colleagues[45] identified distinct Old World and New World subclades after the "California ancestor" branch point, which suggests that although *F. tularensis* subsp. *holarctica* may have emerged in Asia, the massive radiation within this group commenced in North America. How it has moved

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among continents is yet to be discerned, although it seems possible that humans may have played a role, similar to the dispersal of the *F. tularensis* subsp. *tularensis* A.I., which was transported to the eastern United States via of the transport of infected rabbits [40, 46]. *F. tularensis* subsp. *holarctica* seems to occupy more diverse ecological niches that provide for enhanced long-term survival in soil and water, perhaps better facilitating long range dispersal.

The ancestry of individual subspecies is unclear as very little is known about the identity of the near neighbors of *F. tularensis* 37. Like *F. tularensis* itself, they can be difficult to find, sample, and grow in the laboratory. However, non-culture based sampling of the environment finds evidence for widespread existence of *Francisella* spp., many of which are probably nonpathogenic but nevertheless could have important evolutionary ties to the pathogenic *F. tularensis* subspecies[47]. Indeed, these cryptic near neighbors may represent the ancestral states for each of the more apparent and pathogenic *F. tularensis* subspecies. Given the phylogenetic structure and many non-culturable examples within *Francisella*, it seems likely that *F. tularensis* itself may even contain additional subspecies that are currently unknown.

Despite significant effort, the overall ecology of *F. tularensis* is not well understood, particularly transmission cycles [37] and ecologic differences among the different subspecies and groups. It has been identified in more than 300 species of mammals, birds, amphibians, and invertebrates [48], which makes identification of specific transmission cycles difficult [37]. However, in general, it appears that human cases are most often associated with exposure to lagomorphs, rodents, and blood-feeding arthropods [37]. *F. tularensis* subsp. *holarctica* infections (Type B) are also associated with rivers, streams, and flooded landscapes[37], which may represent persistence of the bacterium within protozoans [37, 49, 50]

Synthesis and General Principles

Emergence

B. anthracis, *Y. pestis*, and *F. tularensis* are all quite young species, as suggested by their monomorphic population structures (Figs. 1-3). How then did these species arise and spread globally so quickly? Although less clear for *F. tularensis*, each of these species appears to be the result of a single or few evolutionary changes from their respective ancestors. Many other genetic variants were undoubtedly generated in the ancestral populations but these are the variants that were successful. It seems likely that one of the keys to the success of these clonal species is their increased virulence compared to their ancestors, which was facilitated by lateral gene transfer but also perhaps by genome reductions. This facilitated a shift into much more restricted niches compared to the broad niches of their environmental ancestors. Specialization into restricted niche may help to reinforce their clonality by limiting access to lateral gene donors. Of great importance, this pattern is repeated in highly successful clones found within each of these three species; these successful clones account for most of the global occurrences of each of these pathogens. Changes in virulence also may be related to success of these clones, as differences in disease manifestation and severity are documented among clades of *F. tularensis*[41] and *Y. pestis*[51], though not yet for *B. anthracis*.

Spread

Many of the phylogeographic patterns observed in these three bacterial pathogens are striking in their similarity, but there are some important differences as well. All three species contain monophyletic and highly clonal clades, representing subspecies or subpopulations that have successfully expanded across multiple continents in the recent past. These events have occurred within an overall population genetic structure in which some clades have remained geographically isolated, while the fit clones have broadly expanded across the globe. Of these three diseases, historical documentation exists only for the spread of plague due to its

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particularly catastrophic effects on human civilization. The third plague pandemic, caused by the 1.ORI group of *Y. pestis*, is well documented and the genomic variation and global distribution patterns within this group are highly consistent with 150 years of evolution. Low levels of variation also are observed in *F. tularensis* subsp. *holarctica* and in the *B. anthracis* “A” radiations, suggesting that these two lineages represent comparable recent expansions. The exact time scale is more difficult to discern due to the differential rates of evolution in the spore-forming *B. anthracis* and the lack of historically documented epidemics in either of these pathogens. However, their phylogeography documents similar clonal expansions and genotype successes. Plague and anthrax expansions have been successful in both the northern and southern hemispheres but, oddly, *Francisella* has been restricted to North America for *F. tularensis* subsp. *tularensis* and to the Northern Hemisphere for *F. tularensis* subsp. *holarctica*. The mode of intercontinental transport of *F. tularensis* subsp. *holarctica* is unclear and, hence, our understanding of this perceived restriction in the distribution of *Francisella* is limited.

For recently emerged pathogens, humans themselves are one of the most important parts of a dynamic environment. The third plague pandemic became a global phenomenon because of the advent of efficient steamship transportation that spread infected rats and flea vectors among continents. Anthrax-diseased animals can walk only short distances before succumbing to death. *B. anthracis* spores, however, have been transported by humans across great distances on the hair, hides, and bones of anthrax killed animals [12]. In all cases, the genetic changes that may have been selected for had to match with the dynamic environment, which clearly included human-mediated long distance transport of highly fit clones, in two of these three diseases, to open niches in order to increase genotypic fitness.

Establishment

Why are these species able to establish foci in some regions of the world but not others? Again, *F. tularensis* is problematic because the overall ecology of this organism is so poorly understood, but several insights can be provided by examining the ecology of *B. anthracis* and *Y. pestis*. As described above, the presence of specific types of rodents appear to be required for the dispersal and/or establishment of *Y. pestis* in new regions, but other factors also may be important. The distribution of *Y. pestis* includes much of the western United States but stops roughly at the 100th meridian possibly due to climate differences. Recent modeling efforts, based upon climate and other remotely-sensed data for the United States, suggest the predicted ecologic niche of *Y. pestis* is limited to the western United States [52]. For *B. anthracis*, the presence of ungulates is probably required to maintain foci. This is consistent with patterns from Australia where anthrax now occurs somewhat regularly in domestic cattle. Populations of *B. anthracis* in Australia, which lacks a native ungulate host, probably were introduced from India around 1850 [12, 53], coinciding closely with the introduction of suitable domesticated ungulate hosts. Specific soil properties also may be important to the long-term survival of spores and, hence, establishment and survival of *B. anthracis* in new disease foci [7]. Together, these patterns suggest that both abiotic and biotic factors are important for the survival and establishment of these pathogens in new geographic regions. Understanding these complex requirements and using them to predict other regions of suitability for these pathogens is challenging, but can be accomplished using approaches such as ecological niche modeling [52, 54-56], which also can be used to examine how the distribution of these species may change under predicted global climate change scenarios [52].

Adaptation

Highly fit pathogen clones result from an interaction between the pathogen and its environment, including hosts. The acquisition of a genotypic advantage can be in the form of a plasmid, phage, gene, or even a single SNP that then dramatically increases the fitness of a bacterium.

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A mutational change alone, however, is not sufficient if the environmental conditions do not allow that genetic change to confer a selective advantage.

Adaptation for dispersal regardless of virulence could be the key fitness factor in clone success. It is commonly assumed that recently emerged pathogens are able to expand into a novel niche due to some newly acquired genetic adaptation. Although research frequently focuses on virulence, many other pathogen phenotypes are important. Spore stability in *B. anthracis*, for example, is a highly regulated trait that affects long-range transmissibility. Spore characteristics could be adaptive traits affecting dispersal and transmission rates. These long-range dispersal events, coupled with adaptations for dispersal, provide pathogens with access to naïve host populations, which is a great fitness advantage for the fortuitous clones. As these new areas may contain similar ecologic conditions to the ancestral regions, pathogens invading these areas are not necessarily occupying completely novel niches but rather occupying the same or similar niches to those found in the ancestral regions. These areas can be thought of as open niches, as the pathogens of interest did not previously have access to these locations and they were unoccupied by other pathogens. In the case of *B. anthracis*, *Y. pestis*, and *F. tularensis* this capacity to invade new areas is facilitated, at least in part, by their ability to infect a wide variety of hosts (*i.e.*, wide niche breadth). Doubtlessly, open niches, access to naïve hosts, rapid global transportation, and the dynamic human-nature interface will create new emerging infectious diseases in the coming years. Anthrax, plague, and tularemia are models for other pathogen population dynamics in our future.

Potentially genetically fit pathogens waiting for an opportunity to reach the global stage can be thought of as “Hopeful monsters”[57, 58]. These fit pathogen clones have emerged and dispersed widely across the globe in pandemics associated with many different diseases. The three diseases discussed here are no exception: *B. anthracis* has the A-clade radiation; *Y. pestis* has the global spread of 1.ORI clade during the third pandemic; and *F. tularensis* has the *F. tularensis* subsp. *holarctica* expansion. The supposition is that these clones have genetically-based attributes that makes them more fit and, therefore, better suited for a global expansion. (This supposition is supported in some examples, but unknown and unproven in most.) If they are genetically superior, why didn't they emerge previously or later? We believe that humans with our ancient migratory patterns and more recent global transportation networks are a paradigm-changing development that dramatically alters the fitness of pre-existing genotypes. Humans provide an opportunity for pathogens to both shift niches, using their genetic adaptations in novel environmental contexts, and obtain access to open niches. Just as macro-evolutionary biologists have been surprised by apparently rapid evolutionary change in their study species, these highly fit clones appear on the global stage as if from nowhere but in reality have developed in isolated foci and just stood ready for emergence.

Text Box 1

Plasmids

The acquisition of novel virulence plasmids by the respective ancestors of *B. anthracis* [1] and *Y. pestis* [2, 3] were key events leading to the emergence of these two species because genes encoded on the plasmids allowed these organisms to occupy new ecological niches. *B. anthracis* possesses two virulence plasmids, pXO1 (~182 kb) and pXO2 (~95 kb) [4, 5]. Important virulence determinants on pXO1 include the genes *cya*, *lef*, and *pagA* [5]. Once *B. anthracis* spores are inhaled by an ungulate host and then convert to vegetative cells, the combined products of these three genes produce the edema and lethal anthrax toxins that eventually kill an infected host, leading to the return of *B. anthracis* to the soil in spore form and thereby completing the life cycle. The pXO2 plasmid harbors genes (*capB*, *capC*, and *capA*) important for the synthesis of the antiphagocytic poly-D-glutamic acid capsule [5]. Most *Y. pestis* strains typically possess three virulence plasmids [6, 7]. Two of these plasmids,

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pPCP1 (or pPst; 9.5 kb) and pMT1(pFra; 100-110 kb), are only found in *Y. pestis*, whereas pCD1 (pYV; 70 kb) also is found in *Y. pseudotuberculosis* and *Y. enterocolitica* 3. pPCP1 carries the plasminogen activator (*pla*) gene, which is important for flea-borne transmission because it encodes a surface protease that allows *Y. pestis* to disseminate away from the site of the flea bite to other areas within the host [7, 8]. pMT1 contains a gene (*ymt*) that enables *Y. pestis* to colonize the gut of flea vectors [9], as well as the *caf* gene cluster, which encodes the fraction 1 (F1) capsule [10]. pCD1 contains genes that encode both a type III secretion system and the *Yersinia* outer membrane proteins (Yops) that are secreted into host cells by this system, which together facilitate survival and replication of *Yersinia* spp. in mammalian lymphoid tissues [11]. The genome of *F. tularensis* contains no plasmids [12].

Text Box 2

Plague Pandemics

It is widely accepted that there have been three major plague pandemics during the course of human history that may have resulted in as many as 200 million deaths. The first pandemic, known as the Justinian Plague, occurred from approximately 541 to 750 A.D. and affected Arabia, central and southern Asia, Europe, and north Africa. The second pandemic, which included the infamous epidemic known as the Black Death (1347-1351 A.D.), started around 1347 A.D. and lasted into the 17th century. The third pandemic likely started in the Chinese province of Yunnan in 1855 and last well into the 20th century [18]. Some researchers question whether *Y. pestis* was the causative agent of the first two pandemics (see discussion and citations in [59]), but compelling molecular data suggest that plague was the cause of at least some of mortality during these pandemics [28, 29, 60, 61]. *Y. pestis* was definitely the causative agent of the third pandemic, specifically strains from the 1.ORI group [25].

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Glossary of Terms

Clonal organisms	Organisms that only reproduce by the division of somatic cells without gene transfer or recombination. In this situation, genetic diversity is generated by mutation alone, and not by gene transfer or recombination.
Fixation	This is a genetic phenomenon in which a previously polymorphic feature takes on the same state in every individual in a population and, hence, is no longer polymorphic.
Horizontal gene transfer	The movement of DNA from one organism's genome into another's. Three primary mechanisms are documented: 1) transformation involves uptake of naked DNA from the environment by a bacterium, 2) phage transduction can facilitate the transfer of DNA, and 3) some bacteria have mating mechanisms for transfer of DNA via conjugation. This word usage is frequently synonymous with lateral gene transfer or recombination.
Homoplasy	The occurrence of same character states in two organisms that is not due to shared descent but rather to other forces, such as a reverse mutation or recombination.
Host shifting	A phenomenon where pathogens acquire the capacity to infect a new host species and perhaps become adapted specifically to that new host.
Mutation rates	Frequency that nucleotide changes occur in a genome, usually standardized by events per generation.
Neutral genetic variation	Polymorphic genetic features that have no impact on the survival or reproductive success of the organism.
Obligate pathogen	An organisms that is only able to survive in a host and/or vector and cannot persist directly in the environment.

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Opportunistic pathogens	Organism that are normally benign but given the right situation (e.g., an immuno-compromised host) can cause disease. Often humans, or other hosts that are occasionally infected by these organisms, are not important to the overall lifestyle of these pathogens but rather are dead-end hosts.
Phylogenetically informative characters	These are polymorphic features (e.g., a locus possessing multiple alleles) that have a character state (e.g., an allele) shared by two or more members (e.g., isolates, strains) of the study group (e.g., a population or a species) in a fashion that leads to insights into the evolutionary relationships among members. An example of a non-informative character would a character state that is found only in a single member of the study group. In this case, one knows that that member is unique but this character provides no information about how it is related to other members of the study group.
Phylogeny	A model of the evolutionary history of a set of individuals, species, or other taxonomic units. Phylogenies should be thought of as hypotheses that are based upon analysis of phylogenetically informative characters that arose as the organisms evolved. Phylogenies are traditionally used to understand the evolution of species and other taxonomic units, but are applicable to clonally propagating populations as well.
Phylogeography	The combination of two disciplines, phylogenetics and geography, to understand the geographic distribution of evolutionary patterns in a given organism.
Pleiotropic mutation	A single genetic change that affects multiple phenotypes. For example, a regulatory mutation may impact many different genes and thereby the multiple phenotypes associated with those different genes.
Recently emerged pathogen	A pathogen species, subspecies, population, or genotype with a short evolutionary history. They emerge from another species or population possibly, but not always, due to a genetic change (mutation) that increases their fitness leading to an increase in their frequency and distribution. The recent emergence of such pathogens is often inferred based upon a relative lack of genetic diversity among individual isolates.
Substitution rate	The speed at which a previously polymorphic feature becomes fixed or identical in all members of a population or species.
Sylvatic	Having to do with wild animals, as opposed to commensal animals, which are associated with humans.

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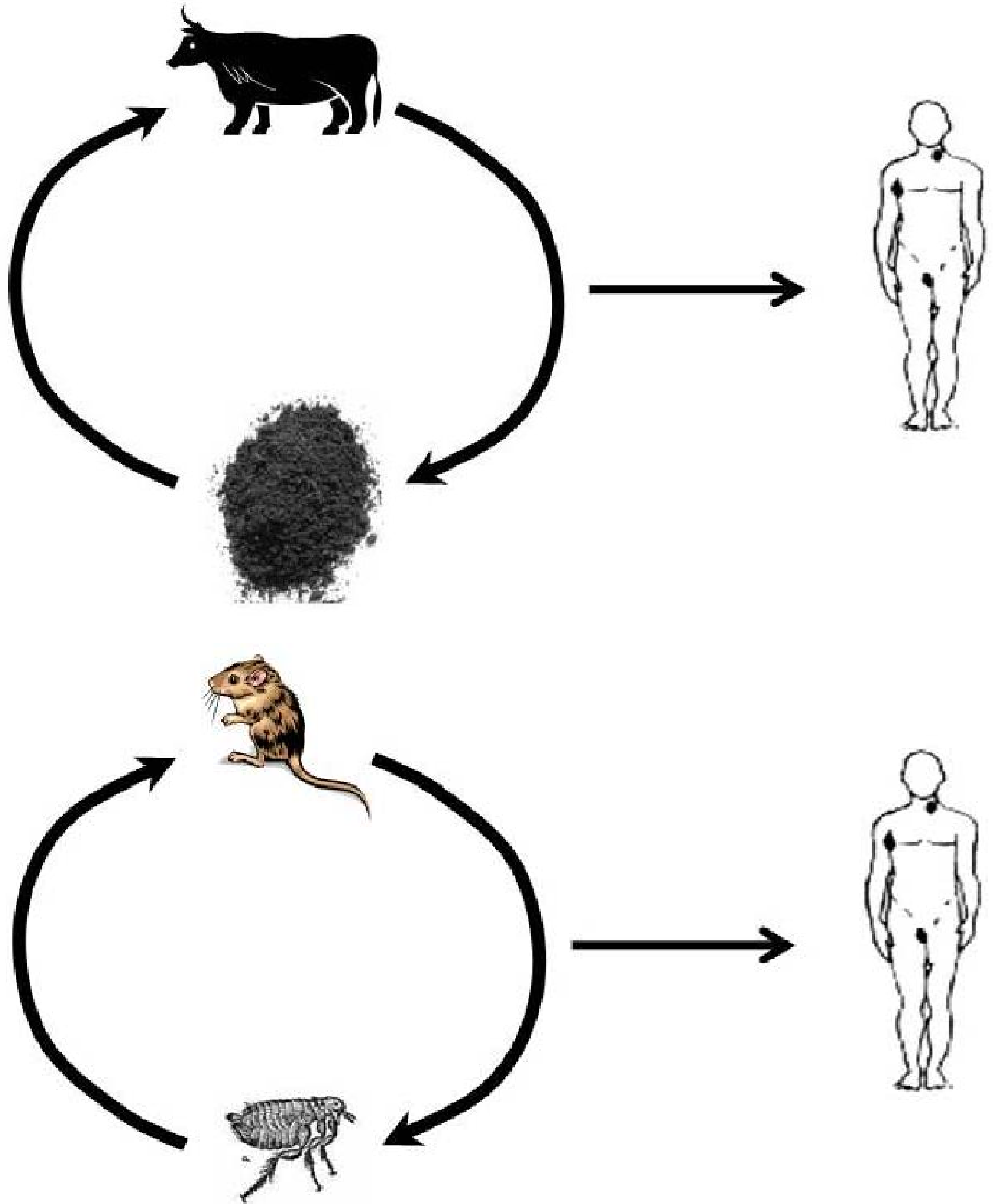

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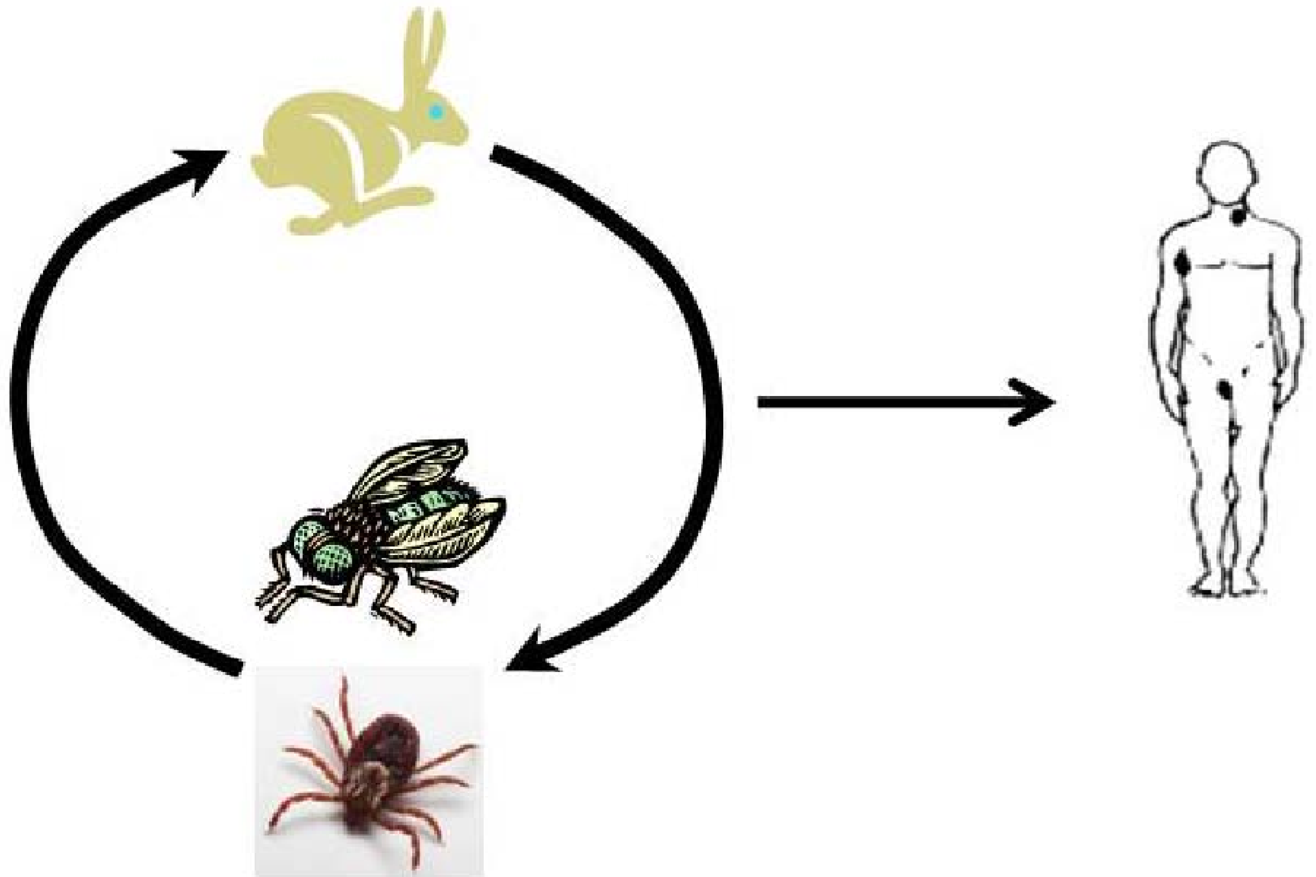

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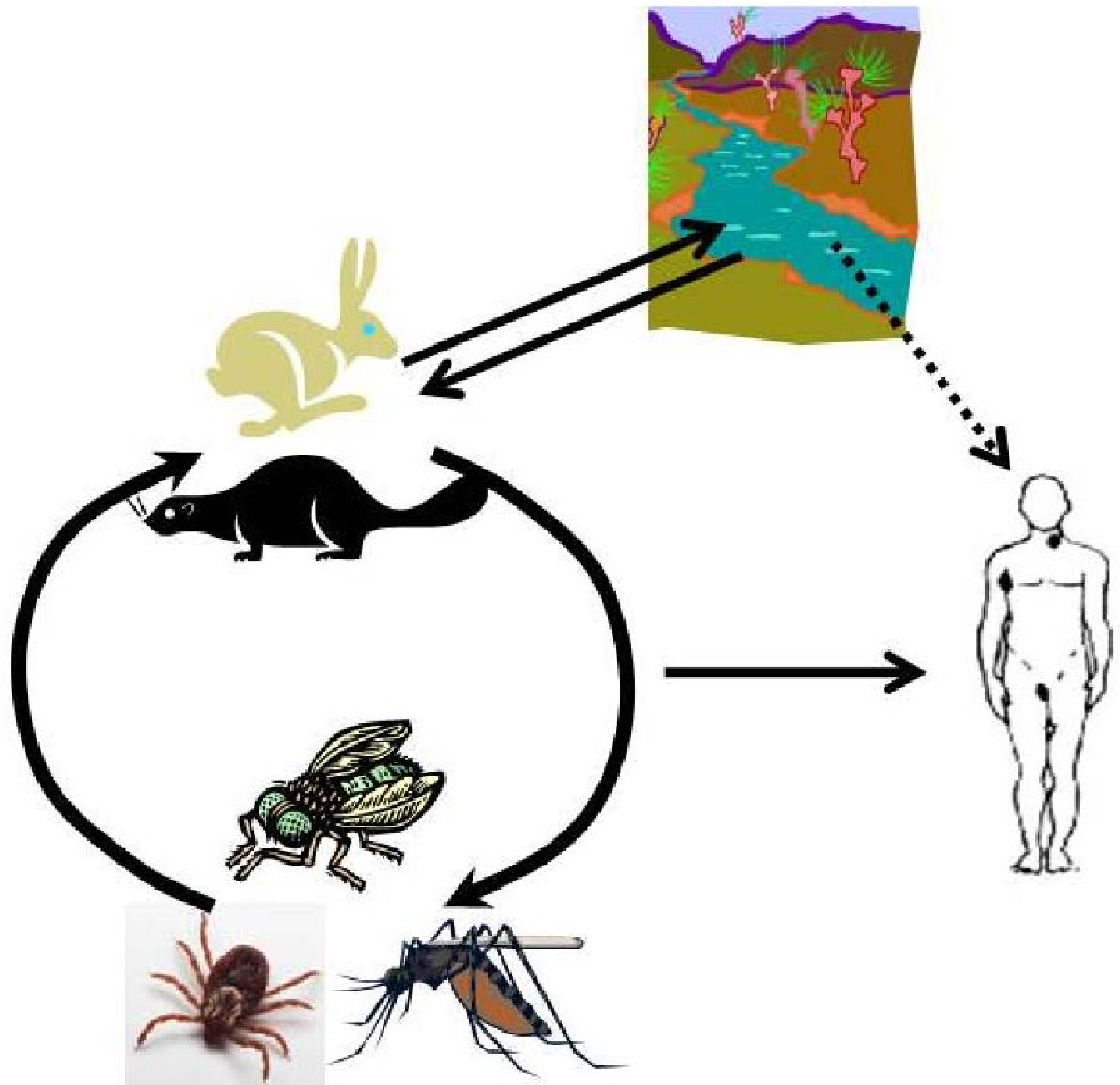

Figure 1.

Figure 1a. Infection cycle of *Bacillus anthracis*. Cattle and other ungulates serve as hosts for *Bacillus anthracis*, which causes anthrax. They inhale spores from the soil while grazing and, once ingested, these spores germinate. The bacteria then ultimately kill the host. Humans can be exposed to spores from the environment or contaminated animal products, such as meat or skin.

Figure 1b. Infection cycle of *Yersinia pestis*. Rodents are the hosts of *Yersinia pestis*, the causative agent of plague, and fleas are the vectors that spread the organism between hosts. *Y. pestis* continuously cycles between hosts and vectors without ever persisting in the

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environment. Humans are most commonly infected from the bite of an infected flea but can also become infected through contact with an infected rodent or other host.

Figure 1c. Infection cycle of *Francisella tularensis* subsp. *tularensis*. Type A tularaemia, caused by *Francisella tularensis* subsp. *tularensis*, is thought to be primarily a disease of lagomorphs (rabbits and hares) and arthropods. Lagomorphs are the hosts for the bacterium, and ticks and flies serve as vectors that transmit the disease between infected and naive hosts and may also serve as long-term reservoirs for the bacteria. Humans are typically infected by the bite of an infected tick or fly or by handling a diseased animal.

Figure 1d. Infection cycle of *Francisella tularensis* subsp. *holarctica*. *Francisella tularensis* subsp. *holarctica* has a more complex life cycle. A variety of mammals are thought to serve as hosts, including hares, rabbits and beavers. Vectors include blood-feeding mosquitoes, tabanid flies and ticks, and ticks are also possibly long-term reservoirs for the pathogen. Water probably serves as one route of infection for mammals, which may seed aquatic areas. Humans become infected primarily by contact with infected hosts or vectors, but water may also be a source of infection.

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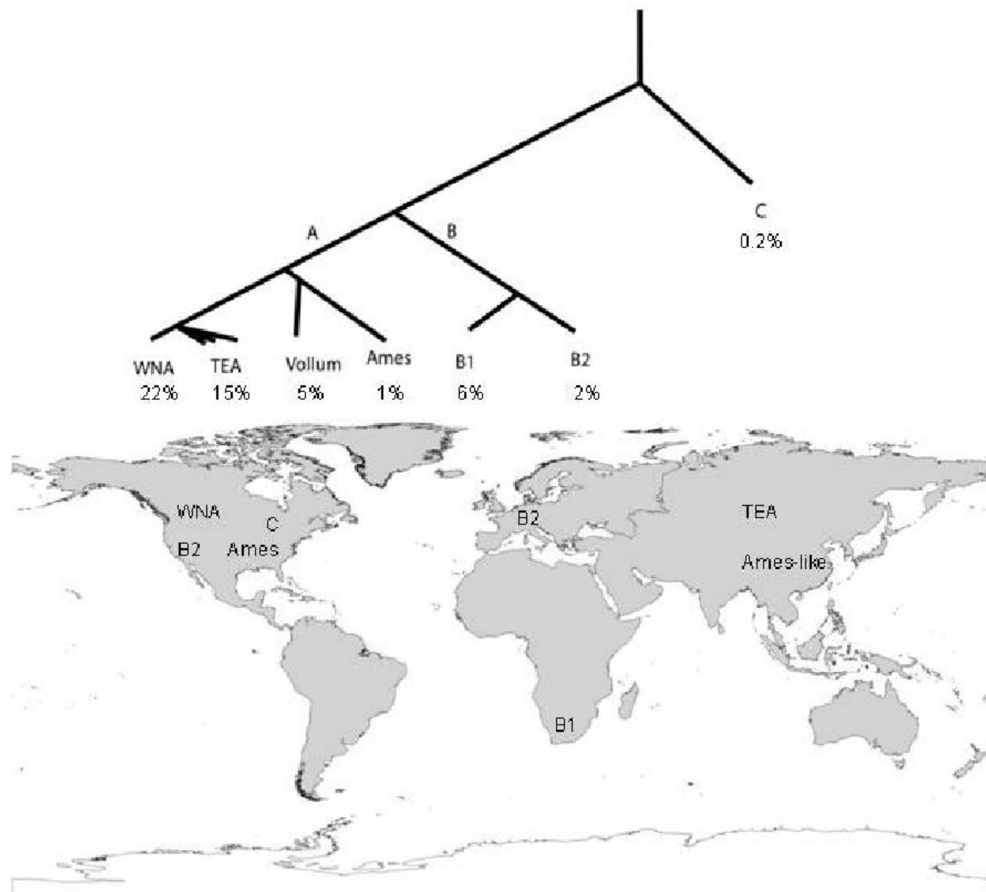

Figure 2. Phylogeography of *B. anthracis*

The population structure of *B. anthracis* revolves around three major groups (A, B, and C). The A group is found in all parts of the world and is very common, whereas the B1, B2, and C groups are relative rare and mostly restricted to subcontinental locations. Highly successful clonal lineage exist even within the A-radiation.

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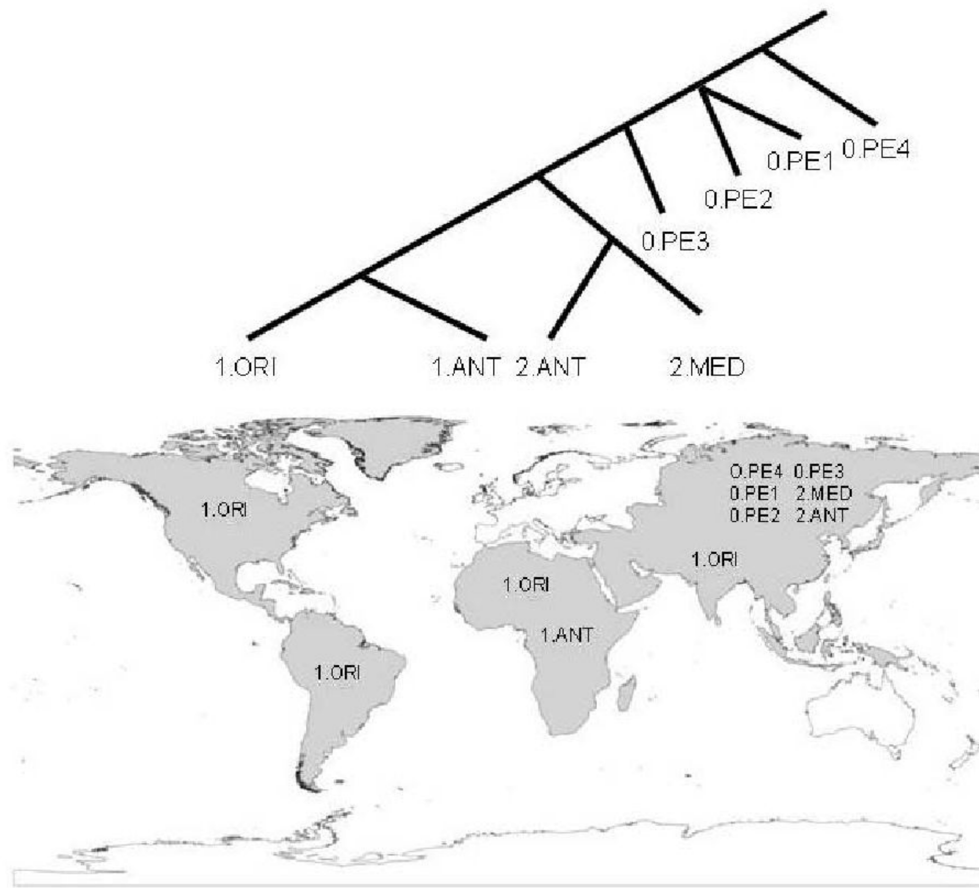

Figure 3. Phylogeography of *Y. pestis*

There are currently three major branches within the *Y. pestis* phylogeny (0, 1, and 2), with eight molecular groups. Six of these groups are only found in Central Asia, where *Y. pestis* likely evolved from its *Y. pseudotuberculosis* ancestor. The 1.ANT group is found in just a small region of Africa. The 1.ORI group is widely distributed and currently occurs on all continents except Australia and Antarctic. This group was spread around the world within the last 150 years by humans during the third plague pandemic.

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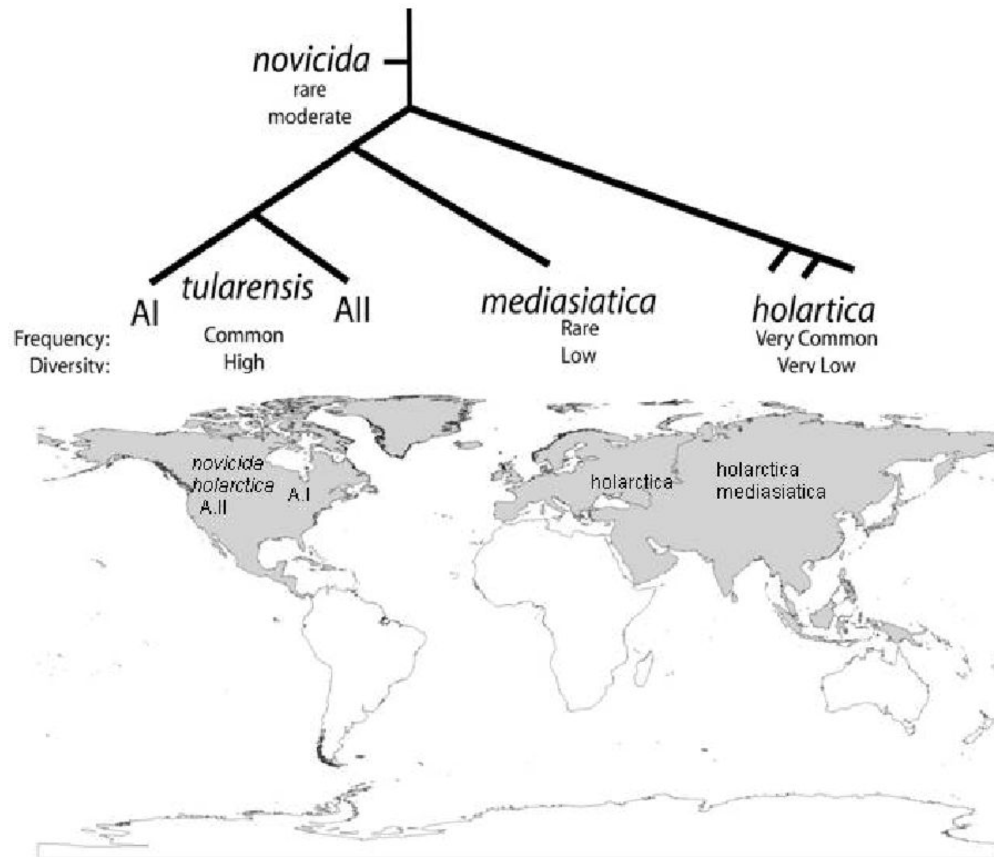

Figure 4. Phylogeography of *F. tularensis*

The subspecies *of F. tularensis* are clonal, with differential success on a global scale. *F. t.* subsp. *tularensis* is differentiated into two subpopulations locally restricted to parts of North America. The subspecies *novicida* and *mediasiatica* are the rarest and restricted mostly to North America and Central Asia, respectively. *F.t.* subsp. *holarctica* appears to be highly fit and highly mobile with a circumpolar distribution in the northern hemisphere.