

Conflict of Molecular and Ecological Phylogenies of the Plague Microbe *Yersinia pestis*: A Search for Consensus

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Abstract—The introduction of molecular genetic (MG) methods into the study of the causative agent of plague, the microbe *Yersinia pestis*, has led to two important discoveries in the problem of reconstructing the phylogenetic history of this apocalyptic pathogen. Its direct ancestor was identified as the causative agent of intestinal infection, Far Eastern scarlet-like fever (FESLF, *Y. pseudotuberculosis* 0:1b), and the time of divergence from the direct ancestor was established at no earlier than 30000 years ago, in the Late Pleistocene or Holocene. However, the molecular methodology does not allow us to create a sufficiently substantiated scenario of the origin and global expansion of the plague microbe. The MG conclusions are not consistent with the facts provided by other natural sciences, primarily ecology and biogeography. At the same time, these discoveries made it possible to create a consistent ecological (ECO) scenario and propose a phylogenetic scheme reflecting the processes of speciation and intraspecific diversification of the plague microbe, which can become a visual evolutionary model for improving the phylogenetic methodology.

Keywords: *Yersinia pseudotuberculosis*, *Yersinia pestis*, *Marmota sibirica*, speciation, phylogeny, phylogeography, evolutionary models

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The biological and taxonomic diversity of microbial pathogens is the result of historical development; therefore, it is impossible to understand fully the properties of current pathogens and to develop methods and techniques for the diagnosis, treatment, prevention, and forecasting of epidemic complications without knowing their history. This circumstance elevates historical reconstructions of infectious agents, i.e., reconstructions of their phylogenies, to a priority task of medical and biological science. The last one and a half to two decades have been characterized by the rapid development of molecular genetic (MG) research methods in bacteriology, which have covered deeper levels of life organization—genetic and molecular. But it should be understood that modern, both classical and MG, methodologies of phylogenetic constructions are not devoid of subjective components. The topologies of the proposed phylogenetic schemes depend to a certain extent on the views and methodological choices, preferences, and assumptions of the authors—researchers. Therefore all created phylogenies are nothing more than hypotheses, among which there may be both more or less convincing ones. As a consequence, phylogenies created by different authors and using different approaches and methodologies are often incompatible, conflicting (Abramson, 2013; Keating et al., 2023).

Concerning the plague pathogen *Yersinia pestis*, targeted attempts to elucidate its evolutionary trajec-

tory were undertaken within the framework of the Theory of Natural Focality of Plague (TNFP, sylvatic plague, wild rodents plague), developed in the mid-20th century. These attempts relied on a limited set of biochemical, ecological, and paleobiological data, analyzed using relatively primitive empirical—intuitive methods. The introduction of modern genetic and molecular methods over the past 15–20 years has elevated the diagnostics of this pathogen to a high degree of precision, enabling clear characterization of its diversity at the subspecies, genovariant, and individual strain levels across the vast majority of known natural and anthropogenic plague foci worldwide. The revealed genetic and molecular diversity has become the material foundation for the contemporary MG approach in phylogenetic reconstructions of *Y. pestis*.

By the end of the second millennium, the TNFP was enriched with diverse new medical and biological data, including ecological data (ECO), which expanded knowledge about the hosts and carriers of the plague microbe and about the microbe itself, which conflicted with some previously proclaimed “classical” assumptions and provisions of the TNFP, as well as with some conclusions of molecular and genetic studies in the MG approach (Suntsov and Suntsova, 2000; Suntsov, 2022a; Suntsov, 2023b). Thus, two approaches were defined in solving the problem: MG and ECO. Within the framework of these approaches, it became possible to evaluate,

compare, and contrast individual conclusions and complete scenarios that reveal the history of plague at different levels of organization of living systems: molecular, genetic, organismic, population, and ecosystem. New molecular, genetic, and ecological (in a broad sense) facts and data have allowed the creation of numerous and diverse phylogenetic schemes of the plague microbe, but, on the other hand, have required the search for a phylogenetic consensus: any history is invariant. This article examines some contradictions in the molecular, genetic, and ecological conclusions on the phylogenetics of the plague microbe with the aim of finding a consensus solution. The primary focus is on substantiating the root of the *Y. pestis* phylogenetic tree and analyzing the habitats of ancestral and derived microbial species that contributed to the formation of this root. The MG approach pays little attention to this issue, despite the fact that habitat is the primary factor of natural selection and, consequently, speciation. Therefore, when reconstructing the history of *Y. pestis*, knowledge of the habitat (the rodent–flea parasitic system) in which the interspecies transition from the ancestor occurred, along with a clear understanding of the population–genetic mechanisms underlying this transition, is an absolute necessity. Without this, selecting the correct evolutionary model—and, accordingly, constructing reliable phylogenetic schemes—is impossible. The imperative to employ ECO methodology in reconstruction of the history of plague is driven by two key discoveries made within the MG approach at the turn of the 20th–21st centuries.

TWO DISCOVERIES OF THE MG APPROACH

The introduction of MG methods in the study of the plague pathogen has yielded, in addition to describing intraspecies diversity and enabling rapid differential diagnosis, two fundamental theoretical discoveries. Firstly, the study of the O-antigen and conservative genes (*thrA*, *trpE*, *glnA*, *tmk*, *dmsA*, *manB*) responsible for the functions of general metabolism in pseudo-tuberculosis and plague microbes has established that the indisputable direct ancestor of the plague microbe is the pathogen of intestinal infection, pseudotuberculosis, more precisely the first serotype of this pathogen (*Y. pseudotuberculosis* O:1b), which causes Far East scarlet-like fever (FESLF) (Somov, 1979; Somov et al., 1990; Skurnik et al., 2000; Fukushima et al., 2001). FESLF is widespread in cold regions of Asia, including Siberia, the Far East, and Central Asia. The FESLF pathogen is a psychrophilic saprozoontic microorganism that has two habitats: external nonliving organic matter (saprophytic phase) and the digestive tract of a wide range of invertebrates and vertebrates (zoonotic phase). In this case, a warm-blooded host can become infected only after the pathogen has been in the saprophytic phase for a long time (Somov and Varvashevich, 1984). It became clear

that the speciation of the plague microbe from the clone of the FESLF pathogen took place in some cold region of Asia and that only the “cold-loving” non-host saprophytic free-living phase of the life cycle capable of infecting warm-blooded hosts could be responsible for this evolutionary event: the FESLF pathogen cannot be transmitted directly from one warm-blooded animal to another. Secondly, the “molecular clock” showed the time of divergence of the FESLF and plague pathogens no earlier than 30000 years ago, i.e., in the Late Pleistocene and/or Holocene; more often the time range of 5000–7000 years ago is indicated (Achtman et al., 2004; Morelli et al., 2010; Cui et al., 2013). Thus, it became clear that the speciation process took place in an (almost) modern biogeocenotic environment, when modern species and subspecies of rodents—hosts of the plague microbe and fleas—vectors and arid landscapes in their (almost) modern boundaries already existed. The two discoveries mentioned led to a revision of the “classical” provisions of the TNFP and initiated the formation of a new, ecological view on the history of the plague pathogen, now as an evolutionarily “young” species that arose (almost) before our eyes.

PHYLOGENETICS OR PHYLOGEOGRAPHY?

Molecular phylogenetics with its advanced methodology absolutely dominates in reconstructions of the history of taxa. The subject of study of phylogenetics are taxa of high rank, species and above. The history of such taxa almost always goes back to geological times, amounting to hundreds of thousands and millions of years, and the original species, the ancestors of the studied groups (root taxa), and even more so the original subspecies (populations), are almost always unknown. Therefore, for the reconstruction of the history of higher taxa, complex computer-statistical technologies are developed that allow us to judge the degree of relationship of taxa by the degree of similarity of features (currently mainly DNA markers). There are up to 400 different algorithms and programs used in MG phylogenetic constructions (Keating et al., 2023). The molecular phylogenetics of the plague microbe borrows the techniques of this complex methodology, considering them universal and quite applicable to the causative agent of the plague. However, the subject of historical reconstruction of the causative agent of the plague is the species and its intraspecific groups, subspecies, i.e., lower taxa. Lower taxa are not of central interest to phylogenetics, and its methodology is not “tailored” to the analysis of the features of lower taxa. Moreover, the species *Y. pestis* is unique in the family of the intestinal inhabitants *Yersiniaceae* (*Enterobacteriaceae*), to which it is assigned by taxonomists. The “blood” plague microbe does not share the same evolutionary tendency as intestinal microbes. It has gone through a peculiar, unique path of development as an agent of transmissible infection and does

not obey the general patterns (models) of the evolution of intestinal bacteria (Suntsov, 2022a, 2022b).

In the modern phylogenetics of *Y. pestis*, a paradoxical situation arose. On the one hand, the molecular direction (MG approach) became mainstream, dominating in the study of the history of the plague microbe. On the other hand, exclusively molecular data do not allow ranking the identified intraspecific diversity “by age” and cannot answer the question about the original form (subspecies, genovariant) of the pathogen and its main host: in the populations of which species of burrowing rodent in the recent evolutionary past did the formation of the species *Y. pestis* occur. At the same time, all the species diversity of rodents, the main hosts of the microbe, and the modern intraspecific diversity of the microbe itself have been well studied. A lot of information has been accumulated about the “archaeological” forms of the plague microbe, described from fragments of DNA preserved in the remains of human victims of past plague epidemics (Valtuna et al., 2022). This information, instead of a real ancestor, allowed us to describe only the statistically designated abstract most modern form, the so-called MRCA (most recent common ancestor), which does not carry any population–genetic and general biological information (Cui et al., 2013). The key question remains unanswered: what subspecies or genovariant of *Y. pestis* does this statistical MRCA represent? It is known that the population of the ancestral species gives rise to the population of the derived species, i.e., the population of the FESLF pathogen in the recent past gave rise to the population of *Y. pestis*. Therefore, the question of the MRCA belonging to a certain population (subspecies or genovariant) of the pathogen that circulates in the population of a certain original primary host is the key to the problem. The MG approach does not provide an answer to this direct question. The reference to the extinction of the original population of the plague microbe and its disappearance without a trace is untenable (Stenseth et al., 2022). A suspicion arises that the MG approach either has some serious methodological flaw that does not allow solving this key issue, or this methodology has application limits that prevent the solution of the phylogenetic problem.

The history of the plague microbe, i.e., its phylogeny, is characterized not only by cellular and somatic (biochemical, genetic, MG markers) factors, but also by extrasomatic (in the words of L.P. Tatarinov) factors characterizing the habitat and ecological factors. It is impossible to propose a meaningful evolutionary model of the “blood” plague pathogen, unique among intestinal bacteria, without understanding the action of such factors and events as changes in gene flow, introduction into a new ecological niche, isolation, competition, and environmental stress. The universal neutral model used in almost all modern phylogenetic constructions is logical and consistent, but in the case of *Y. pestis*, it seems reductive or too general. A full-

fledged ad hoc model is needed, which should also include ecological and population–genetic mechanisms for the emergence of the *Y. pestis* isolate and the formation of an interspecies gap between a certain population of the intestinal FESLF pathogen and the population of a direct “blood” descendant; i.e., the model should characterize the mechanism of habitat change or ecological niche change (Suntsov, 2022a, b). And it is not a fact that the neutral evolution model meets such requirements.

It follows from the above that the history of the plague pathogen is an object of study not so much of phylogenetics as of another science—phylogeography. Phylogeography originated in the depths of phylogenetics, but is aimed at a different object of research, studying the history of a species and intraspecific forms. This science has its own methodological features. In addition to MG methodological concepts, it operates with population–genetic and ecological concepts such as the geographic population, subspecies, range, isolation, gene flow, general and local adaptation, behavioral pattern, ecological niche, adaptive radiation, and others (Kholodova, 2009). Due to this, the methodology of phylogeographic studies includes an analysis of not only neutral phylogenetic features, but also adaptive ones, which are given a subordinate place in molecular phylogenetics, which takes the neutral evolution model as a theoretical basis.

THE MG APPROACH

The problem of the plague microbe phylogenesis is acutely directed by the “spirit of the times.” The MG approach prevails so strongly that general biological facts and even established provisions of the evolutionary theory are omitted from the analysis, which casts great doubt on many MG conclusions about the phylogenesis of *Y. pestis*. The fact that MG dendrograms and cladograms are recognized by the majority of (MG) researchers does not mean that they are indisputable when considered from the point of view of other sciences: the patterns of molecular evolution have not yet been revealed in the necessary completeness. In fact, the MG approach itself in the phylogenetics of the plague microbe cannot be considered unified or monolithic. Some genetic and molecular components of this approach cannot be considered congruent (Suntsov, 2023a, 2023b).

The molecular methodology is focused on a comparative analysis of nucleotide sequences in the genomes of the ancestral and derived species and, in general, adheres to the Leibniz principle of gradualism in matters of the development of the world and evolution (“natura non facit saltum”). Its theoretical basis is the model of gradual neutral evolution. Conservative features (molecular markers) of the FESLF pathogen and modern and “archaeological” genovariants/subspecies of the plague microbe are compared and analyzed, the quantitative indicators of which are used to

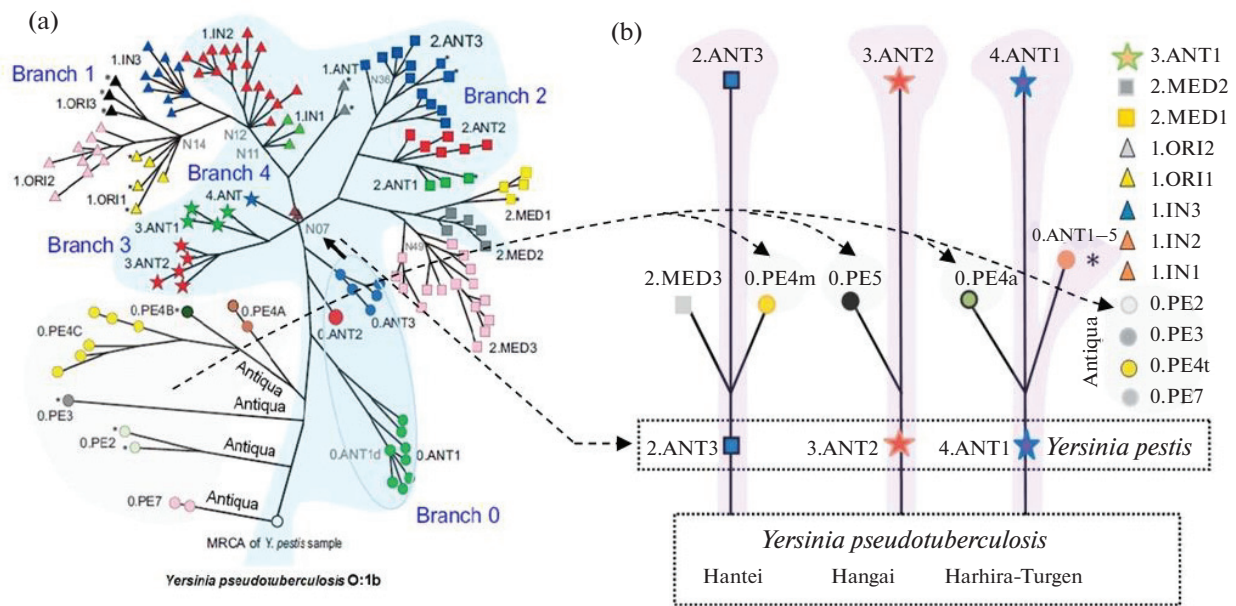


Fig. 1. Phylogenetic trees: (a) molecular genetic (Cui et al., 2013); (b) ecological (Suntsov, 2022b). The solid arrow (Fig. 1a) shows the belonging of the marmot genovariants cluster 0.ANT to the polytomy node N07 (Wu et al., 2022). The dotted arrows show the locations of the polytomy node N07 and the genovariants/subspecies of the “vole” cluster 0.PE on the ecological three-rooted tree of *Yersinia pestis*. The phylogenetic lines of marmot subspecies/genovariants are outlined in the blue background.

judge the degree of their homology and, accordingly, the degree of kinship of the carriers of these features (Morelli et al., 2010; Cui et al., 2013; Cui and Song, 2016). But in reality, the phylogenetic process of the plague microbe has two largely different stages, speciation and intraspecific diversification, and, accordingly, should be represented by two different evolutionary models. Speciation is a unique irreversible microevolutionary process ending with a macroevolutionary effect, the conquest of a new ecological niche and the emergence of a new species, while intraspecific diversification is a “ubiquitous” reversible microevolutionary process of ongoing adaptation of populations to local living conditions. In the first (macroevolutionary) case, Dollo’s evolutionary idea is applicable, according to which evolution is irreversible and the secondary appearance of traits in their original form is impossible. In the second (microevolutionary) case, traits are recognized as completely reversible (Fitch–Wagner model) (Lukhtanov, 2013). In contrast to this, the molecular methodology considers the entire phylogeny of the plague microbe, both macro- and microevolutionary historical processes, as a consistent uniform accumulation of neutral mutations, counted by each genovariant/subspecies from the ancestral form. The number of mutations that have arisen is used to judge the degree of the relationship of the compared strains (taxa): the more nucleotide changes have accumulated, the more distant the relationship. However, it must be assumed that mutations in micro- and macroevolutionary processes have vary-

ing significance and different weight and frequency; hence, the neutral evolutionary model may not adequately reflect the evolutionary reality, especially over evolutionarily short periods of time (Suntsov, 2018, 2019). The molecular “neutral” phylogenetic tree of *Y. pestis* represents the history of the abstract initial form of the MRCA, which first gave rise to the abstract trunk 0 (Branch 0), which in turn gave a powerful irradiation (polytomy node N 07), dividing almost simultaneously (star-shaped) into four derivatives, also abstract branches (Branches 1–4) (Fig. 1a). But the proposed phylogenetic abstractions of the MRCA, trunk, and branches, and the subspecies/genovariants generated by them, “do not fit” the biogeographic map of Eurasia. The topology of the “molecular” phylogenetic tree does not harmonize with the description of the routes of expansion of the plague microbe from the supposed centers of speciation. In short, it is not possible to create a sufficiently reasoned scenario for the phylogenesis of the plague microbe based on molecular markers (Cui et al., 2013; Suntsov, 2023b, 2024).

The genetic methodology is proposed due to the need to somehow show the relevance of adaptive traits in the history of the plague pathogen. The genes of the plague microbe encoding adaptive traits are highly informative for reconstructing the history of the pathogen. This approach proclaims the formation of the plague microbe due to adaptive “horizontal evolution” and adopts the saltation model of evolution: “species-specific adaptive genetic elements” are

“integrated” into the pseudo-tuberculosis cell, ensuring the existence of the microbe in a new environment, and genes that have lost their functions in the new conditions are eliminated/inactivated. Despite the outstanding achievements in the diagnosis of the plague microbe and the description of intraspecific diversity, in matters of speciation and phylogenetic constructions, the modern genetic approach in some respects remained at the positions of early geneticists, who were based in the typological concept of macrogenesis. This concept assumes instant speciation from single individuals that have experienced a “successful” adaptive macromutation, or, in the famous expression of R. Goldschmidt, from the so-called “hopeful monsters.” In relation to the plague microbe, this means that single microbial pseudo-tuberculosis cells enter a new habitat, the rodent-flea system, where new genetic elements (genes, gene islands, specific virulence plasmids pFra and pPst) are introduced into them by horizontal gene transfer and successfully integrated into the genetic apparatus, and non-functional ones (*ureD*, *inv*, PDE 2, PDE 3, *rc* sA) are eliminated/inactivated, radically changing the phenotype (Sun et al., 2014; Hinnebusch et al., 2016). This approach ignores modern concepts of population-genetic mechanisms of evolution and speciation. The fatal error of all theories of saltational evolution is that they operate with mutating individuals, not populations, while it is local populations and not unique individuals that are the key to all questions of evolution. Speciation occurs through unstable transitional population forms in an unstable intermediate environment, which characterizes the gap between the ecological niches of the ancestor and the direct descendant. Saltation concepts are currently given a limited place in evolutionary science; they cannot explain the achievement of species isolation and ecological compatibility with direct ancestors in the overwhelming majority of known taxa. This also applies to the plague microbe.

In assessing the MG conclusions on the phylogeny of the plague microbe, it should be noted that the MG approach does not consider the initial stages and the process of speciation itself and does not discuss changes in the microbial habitat (biogeocenosis) that caused this process, while it is precisely changes in the habitat of a population (species) that lead to (micro)evolutionary shifts and, ultimately, to the formation of a new species. Therefore, it is changes in the habitat of the FESLF pathogen that can more convincingly characterize the evolutionary model used in phylogenetic reconstructions of the plague microbe. The neutral evolution model, “tested” in phylogenetics, is acceptable mainly when considering the history of high-ranking taxa and over long geological periods of time. It is now obvious that the changes in the habitat of the FESLF pathogen that led to the emergence of the plague microbe occurred in the recent evolutionary past (from several thousand to 30000 years ago) and should have left biotic and/or abiotic

“traces” in the corresponding biogeocenosis. To characterize the evolutionary model of *Y. pestis*, these traces are subject to careful study. Changes in the structure of biogeocenoses are studied by sciences such as biogeocenology, ecology, biogeography, and paleobiology.

THE ECO APPROACH

In order to obtain an idea of the unique evolutionary model of phylogenesis/phylogeography of the plague microbe, it is necessary to clarify the specific ecological factors and population-genetic mechanisms that caused the divergence of the plague and pseudo-tuberculosis microbes. First of all, it is necessary to identify the rodent-flea environment in which their divergence occurred in the recent past in the (almost) modern biogeocenotic environment. It is necessary to indicate the original host of the plague microbe. It is the original host that is the key object for creating an adequate evolutionary model. For the factual substantiation of this key object (the type of original host), some consistent postulates and presumptions have been proposed, according to which the original host of the plague pathogen from the more than 30 known main hosts of the microbe in Asian foci can definitely only be the Mongolian marmot (*Marmota sibirica*) (Suntsov, 2017a,b). It follows that, in addition to the MG approach for reconstructing the history of the plague microbe, an additive ecological approach is needed, i.e., in the methodology, in addition to neutral phylogenetic ones, it is necessary to use adaptive ecological (in the broad sense) features. The integration of MG and the ecological approaches is seen as a successful solution to the (phylogeographic) problem of the recent (almost modern) period on the evolutionary time scale of the origin and intraspecific diversification of the plague microbe.

The two above-mentioned discoveries of the MG approach and the large volume of ecological (in the broad sense) data on the causative agents of pseudotuberculosis and plague made it possible to create a presumptive ecological scenario for the origin and global expansion of the plague microbe, which seems less controversial compared to other known scenarios and which can serve as a null hypothesis for further research (Suntsov and Suntsova, 2000; Suntsov, 2017a). Briefly, this scenario appears as follows: the plague microbe *Y. pestis* diverged from the FESLF causative agent, the pseudotuberculosis microbe *Y. pseudotuberculosis* O:1b, in a heterothermal and heteroimmune environment, the parasitic system of tarbagan marmot and the flea *Oropsylla silantiewi* under the conditions of the maximum (Sartan) cooling of the climate of Central Asia 22000–15000 years ago. The reason for the emergence of the new species was a change in the behavior of the host and the vector. Further natural adaptive radiation of the initial population was carried out in Eurasia according to the “oil

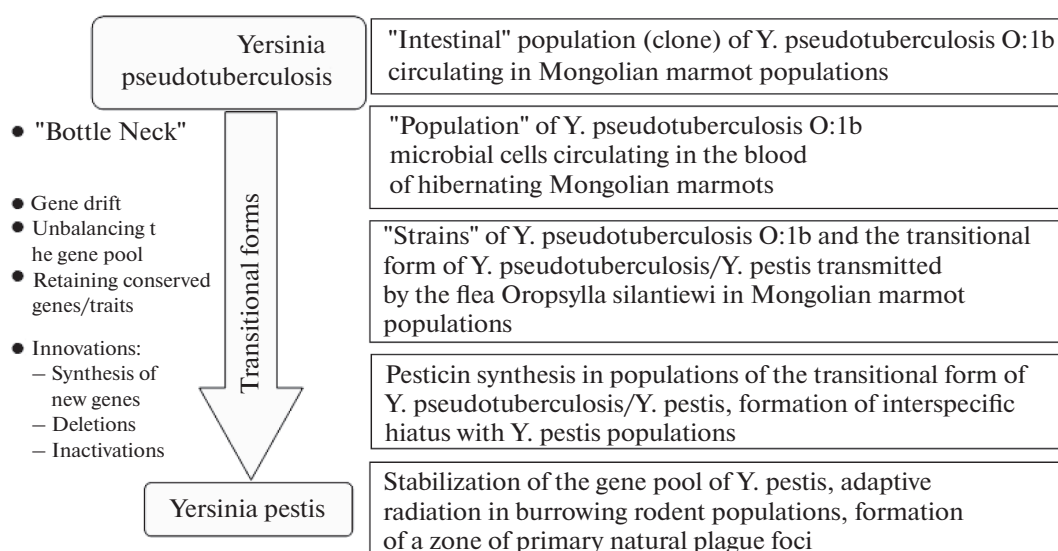


Fig. 2. Reconstruction of the evolutionary transformation of the population (clone) of the causative agent of intestinal infection—the Far East scarlet-like fever (FESLF, *Yersinia pseudotuberculosis* O:1b) into the population of the “blood” plague microbe (*Y. pestis*) in the heterothermal and heteroimmune environment of the Mongolian marmot (*Marmota sibirica*), the flea *Oropsylla silantiewi*. The transition to a new ecological niche was realized by traumatic infection of the Mongolian marmot population with FESLF infection.

spot” principle. The scenario is presented schematically in Fig. 2.

The nonadaptive stable traumatic process in the Mongolian marmot population caused by an abiotic factor (the Sartan cold snap in Siberia) opened a new potentially existing ecological niche for the pseudotuberculosis microbe, into which it managed to penetrate and where it transformed into a new pathogenic species specialized for circulation in the lympho–myeloid complex of the Mongolian marmot, and later in the body of many other burrowing mammals. The ECO scenario proposed as presumptive more than 20 years ago remains not disproved by any new reliable facts. Another possible original host of the plague pathogen, instead of the Mongolian marmot, has not been named. Therefore, the ECO scenario can serve as a presumption for reconstructing the history of the pathogen.

THE MG AND ECO PHYLOGENETIC TREES

As mentioned above, the root, trunk, and branches of the popular MG phylogenetic tree of the plague microbe form abstract forms—MRCA, trunk/branch 0 (Branch 0), and second-order branches 1–4 (Branches 1–4) (Fig. 1a) (Cui et al., 2013). It is believed that the trunk (Branch 0) is formed by the “most ancient” “vole” subspecies of the 0.PE cluster and the “marmot” genovariants/subspecies of the 0.ANT cluster. Of these, the subspecies 0.PE 7, 0.PE 2, 0.PE 3, 0.PE4t, and 0.PE4h have the biochemical property of the “marmot” biovar Antiqua. It is

believed that the biochemical properties have limited phylogenetic significance (Achtman et al., 2004), but, nevertheless, the Antiqua trait may support the idea of speciation of the plague microbe in marmot populations (Anisimov et al., 2016). If this is so, then the “vole” subspecies should be located in the crown and not at the root of the phylogenetic tree of *Y. pestis* (Fig. 1b). “Vole” subspecies most likely do not represent intermediate forms between *Y. pseudotuberculosis* O:1b and *Y. pestis*, as is recognized in the MG approach, but are more advanced, derivative forms that have lost their original property of high virulence in relation to marmots, gophers, gerbils, rats, and humans when adapting to new “vole” hosts.

On MG phylogenetic trees, second-order branches 1–4 form a polytomy node called N07 (Cui et al., 2013) (Fig. 1a). The formation of this node is associated with the (almost) simultaneous diversification of the “classical” strains of the plague pathogen (the main subspecies *Y. pestis pestis*), characteristic of the Tien Shan populations of the Altai marmot (*Marmota baibacina centralis*). Strains from Altai marmot populations (genovariants of cluster 0.ANT), like all “vole” subspecies, belong to the trunk (Branch 0) of the phylogenetic tree and are considered the most ancient among all marmot genovariants/subspecies. At the same time, the estimated time of diversification, according to the “molecular clock,” was within several hundred years preceding the Second Pandemic (“Black Death”), which began in Europe in 1346. The MG approach does not name the reason for such a grandiose, explosive intraspecific diversification of

the plague microbe, which covered vast areas of Central Asia and adjacent territories and which occurred around the time of the reign of Genghis Khan. The hypothesis of a “lightning-fast” anthropogenic Asian expansion of the plague pathogen from the speciation area on the Tibetan Plateau via trade and military routes does not stand up to criticism (Suntsov, 2023b, 2024). Recently, the structure of node N07 was corrected by new data: the “marmot” subbranches 0.ANT, originating from the trunk of the tree (Branch 0), were also assigned or closely approximated to the polytomy node N07 (Fig. 1a) (Wu et al., 2022). It turns out that all “marmot” genovariants/subspecies of the plague microbe biovar *Antiqua* circulating in populations of Central Asian marmots, included in branches 0–4, were formed at almost the same time (on an evolutionary scale). These facts find a plausible interpretation in the ECO approach and may testify in favor of the historical priority of the “marmot” plague.

The ECO scenario provides a detailed picture of the speciation process of the plague microbe in the Mongolian marmot populations in Central Asia and its further global expansion (Suntsov and Suntsova, 2000; Suntsov, 2017a,b, 2018). It is shown that speciation was tritopic and occurred autonomously in three geographic populations of the Mongolian marmot with the parallel formation of three subspecies/genovariants of the plague microbe—2.ANT3, 3.ANT2, and 4.ANT1 (Fig. 1b) (Suntsov, 2021). According to environmental data, the cause of speciation was the last maximum (Sartan) cooling of the climate in North and Central Asia. The cooling led to deep freezing of the soil in all Mongolian marmot settlements in Central Asia and a change in the behavior of the marmot flea larvae *O. silantiewi*. Saprophagous flea larvae from the substrate of marmot nests freezing in the winter months, due to positive thermotaxis, switch to facultative hematophagy on sleeping marmots, which ultimately caused a population-wide traumatic (unique, nonadaptive, but provided by preadaptations, Suntsov, 2017a, 2017b) infection of Mongolian marmot populations with an intestinal infection—FESLF. Adaptation of the population (clone) of the FESLF pathogen to new highly stressful conditions of existence in the lympho-myeloid complex of heterothermic marmots led to the transition to a new ecological niche and adaptive zone and “quantum” speciation—the rapid formation of a new species—the causative agent of the plague, which broke the topical and trophic connections with the digestive tract of the host (Suntsov, 2018).

Despite the obvious differences in the structure of phylogenetic MG and ECO trees, a certain consensus can be seen in them (Fig. 1). From the scheme in Fig. 1a, it is clear that the “vole” subspecies do not constitute a single phylogenetic group. The subspecies of this cluster separated from the phylogenetic trunk at different times, in different geographic areas, presumably, from different populations of the putative

abstract ancestor of MRCA, and formed a para- or polyphyletic group (Kislichkina et al., 2019). Due to their greater specialization in relation to the main hosts, their place in the “crown” of the phylogenetic tree seems more logical (Fig. 1b). At the same time, the “vole” subspecies are located on different phylogenetic “trunks” of the ECO tree; their MG similarity, according to ecological logic, is due to homoplasy, and the relationship is mediated by pseudotuberculous ancestors.

According to the ECO scenario, the polytomy node N07 on the MG phylogenetic tree should be at its base; i.e., the tree topology should be different, without an abstract trunk (Branch 0) and dependent phylogenetic branches (Branches 1–4) (Fig. 1b). At the same time, the rejection of the “trunk” and “branches” does not lead to the loss of information about the phylogeny of the plague microbe. On the contrary, the tritopic speciation of *Y. pestis* and the population thinking in matters of the history of the formation and spread of the plague pathogen in the world can explain many facts that are inexplicable from the standpoint of the MG approach. One such fact, clearly indicating the parallel origin of the plague pathogen in three geographic populations of the Mongolian marmot, is the semisympatry of two pika subspecies of the plague microbe 0.PE4a (*Y. pestis altaica*) and 0.PE5 (*Y. pestis ulegeica*).

SEMISYMPATRY OF THE SUBSPECIES ALTAICA AND ULEGEICA

Nature has bestowed upon researchers of plague microbe phylogeny an invaluable gift in the form of the phenomenon of semisympatry between two subspecies—*Yersinia pestis altaica* and *Yersinia pestis ulegeica* within a single geographical population of the Mongolian pika (*Ochotona pallasi pricei*) (Suntsov, 2023b). This phenomenon provides clear evidence supporting the concept of parallel tritopic speciation of the plague microbe in populations of the Mongolian marmot. The range of the Mongolian pika extends in a broad band from northwest to southeast, from the Altai Mountains to the Gobi Altai, completely encompassing the Mongolian Altai. The essence of this phenomenon lies in the fact that, in the northwestern part of the range (Altai Mountains), only the Altaian subspecies circulates in pika populations, while in the Gobi Altai and southern part of the Mongolian Altai, only the Ulegeian subspecies is found. In the intermediate territory, the northern part of the Mongolian Altai in Bayan-Ulgii aimag of Mongolia, an overlap of the ranges of two subspecies is observed: within the same colony, strains of both subspecies can be isolated from the animals (Fig. 3).

In the sympatry zone, the Altai and Ulegei subspecies of *Y. pestis* behave as established species; i.e., they coexist stably. This is a unique case, as usually any subspecies is adapted to a local (native) habitat and has its

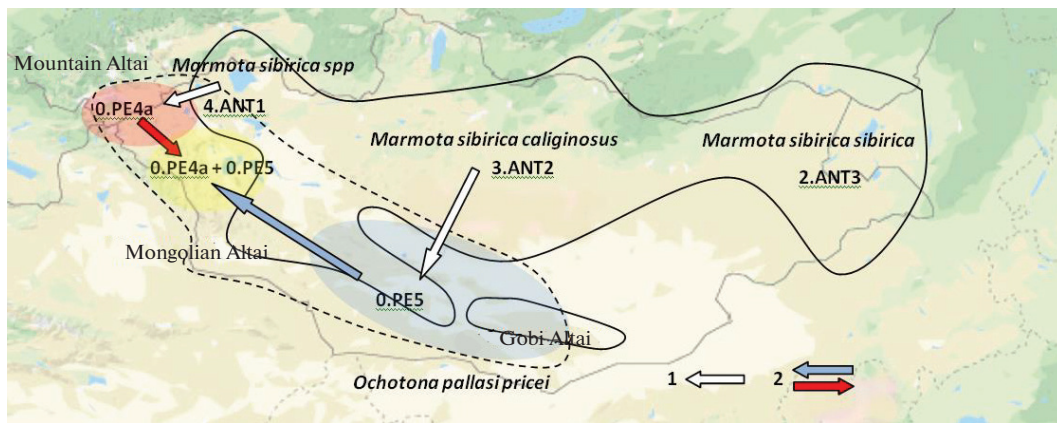


Fig. 3. The zone of sympatry (yellow color) of the subspecies 0.PE4a and 0.PE5 of the plague microbe in the geographic population of the Mongolian pika (*Ochotona pallasi pricei*) in the northwestern part of the Mongolian Altai (Bayan-Ulgii aimag). The red background shows the (presumed) original range of 0.PE4a; the blue background shows the range of 0.PE5. (1) The “transition” of the marmot subspecies of the plague microbe to the pika subspecies. (2) The counter-distribution of the pika subspecies from the areas of their formation in the Gobi and Mountain Altai.

own special, individual ecological subniche: subspecies cannot coexist stably in natural conditions (Mayr, 1963). The observed sympatry indicates equivalent adaptation of different subspecies to the same habitat. Thus, here the same ecological niche (a single monomorphic geographic population of the Mongolian pika, in whose range there are no biogeographic barriers) is occupied by different subspecies of the plague microbe. The reason for this unique case can be explained by the history of the formation and spread of subspecies. Parallel formation of the original marmot subspecies 3.ANT2 and 4.ANT1 led to parallel formation of the pika subspecies 0.PE5 and 0.PE4a, derived from them, in the distal parts of the range of the Mongolian pika, where the Mongolian pika and the Mongolian marmot live together in the Gobi Altai, and the Mongolian pika and Altai marmot, which have areas of sympatry with the Mongolian tarbagan, live in the Altai Mountains. Due to parasitic contacts, the pathogen passed from marmots to pikas; i.e., it penetrated a new ecological subniche and acquired the properties of independent subspecies. Further spread of subspecies 0.PE4a and 0.PE5 in the population of the Mongolian pika along opposite routes led to their interpenetration and the emergence of a modern zone of sympatry in the Bayan-Ulgey aimag of Mongolia. This unique natural phenomenon should become an object of deep study not only in the problem of the phylogenesis of the plague microbe, but also in the problem of speciation and evolutionary theory in general.

CONCLUSIONS

At present, the achievements of MG studies of infectious agents are undeniable. This is especially true for the identification of their biological diversity and disease diagnostics. This also applies to the plague agent, the *Y. pestis* microbe, in relation to which it is

important to note two key theoretical MG achievements, two discoveries made a quarter of a century ago: the identification of the direct ancestor of the plague agent and the establishment of the time of divergence of the ancestral and derived species. These discoveries gave a new impetus to the “classical” studies of the plague phenomenon and initiated the emergence and development of the ECO approach in matters of reconstructing the evolutionary history (phylogeny/phylogeography) of the pathogen. But it so happened that the MG and ECO approaches began to develop separately. The MG approach turned out to be “blinker” by a statistical-computer methodology and does not use the vast “classical” medical and biological material accumulated over the past decades and the past century to evaluate its findings and conclusions. The MG methodology ignores the population approach to the phylogeny of the plague pathogen, deviates from the recognized principles of prokaryotic taxonomy in matters of intraspecific systematics (the taxonomic category of subspecies is interpreted arbitrarily), and despite its “advanced” methodology and well-studied intraspecific diversity, it cannot name the original subspecies of the plague microbe or indicate its rodent host (this is the central issue in the problem of phylogeny/phylogeography of the plague microbe). MG conclusions are sometimes inconsistent with obvious facts and data from “classical” scientific fields; the phylogenetic hypotheses created by MG remain unconvincing.

The conclusions of the ECO approach, supported by many MG facts, seem more consistent. In any case, the ECO scenario, created a quarter of a century ago as a presumptive interpretation of the history of the plague and its pathogen, has not been disproved by any reliable new facts to date. Therefore, the ECO scenario

deserves attention as a verbal evolutionary model in reconstructing the history of the plague microbe.

To summarize this brief review, it should be emphasized that each of the approaches mentioned operates at its own level of life organization and in its own evolutionary context, which is determined by its evolutionary model and has its own advantages and disadvantages. At the same time, each approach makes its own significant contribution to the reconstruction of the history of the plague and its pathogen. But history is always invariant, and in principle there is only one real scenario of the origin and evolution of the pathogen, which must correspond to all possible approaches. It is clear that the conclusions of all approaches must be congruent with each other. “Molecular” and “genetic” histories must not have any contradictions with the “ecological” history. An analytical assessment of various molecular and genetic scenarios in relation to the ecological scenario, which considers the results of evolution at higher levels of life organization, will help to identify the true patterns of molecular and genetic evolution of the plague pathogen. Once molecular and genetic patterns of evolution congruent with other natural sciences, primarily ecology and biogeography, are identified, it will be possible to declare the birth of a full-fledged theory of *Y. pestis* evolution with prognostic properties. This theory will become a reliable evolutionary model for further improvement of the reconstruction of the phylogeny of the plague microbe and, moreover, for revealing some patterns of molecular evolution of pathogenic prokaryotes in general.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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CONFLICT OF INTEREST

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