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THEORETICAL  
AND EVOLUTIONARY BIOLOGY

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# Sympatric Speciation of the Plague Microbe *Yersinia pestis*: Monohostal Specialization in the Host–Parasite Marmot–Flea (*Marmota sibirica*–*Oropsylla silantiewi*) System

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Received November 10, 2014

**Abstract**—An ecological scenario of the origin of the plague microbe that is interpreted in the light of modern Darwinism (synthetic theory of evolution) is presented. It is shown that the plague microbe emerged from a clone of the psychrophilic saprozoontic pseudotuberculosis microbe *Yersinia pseudotuberculosis* O:1b in the mountain steppe landscapes of Central Asia in the Sartan time, 22000–15000 years ago, in the monohostal Mongolian marmot (*Marmota sibirica*)–flea (*Oropsylla silantiewi*) host–parasite system. It was noted that the evolutionary process described corresponds to the sympatric form of speciation by transition of the clone of migrant founders to a new, already-existing ecological niche. It was established that monohostal specialization of the plague microbe was made possible due to heterothermia (5–37°C) of marmots in the hibernation period. The factors of the speciation process— isolation, the struggle for existence, and natural selection— were analyzed.

DOI: 10.1134/S1062359016010155

## INTRODUCTION

At the beginning of the twenty-first century, mankind has had to face new global challenges. Due to the accelerated development of the social–economic and demographic structure of society, rapid anthropogenic environmental changes, and radical transformation of natural landscapes, the problem of emerging and reemerging human and animal diseases is becoming more and more pressing in the world. More than thirty diseases earlier unknown to humans or already-forgotten caused by newly found species and new forms of pathogens, mainly of viral etiology, such as SARS, avian influenza, and Ebola, have been revealed over the last 15–20 years. Moreover, growing social and international contradictions lead to exacerbation of conflicts between the communities of people, including extreme forms such as coup d'états, revolutions, wars, and terrorist acts, among them those with the use of biological agents, i.e., microorganisms causing very dangerous infectious diseases. These alarming circumstances of modernity and the expected destructive challenges in the near future require the development of a reliable system of biological security as soon as possible. In such a case, the knowledge of the principles, mechanisms, forms, and modes of speciation of pathogenic microorganisms and the ways of formation of the properties of their natural high virulence and pathogenicity is becoming an necessary requirement as a theoretical support for the elaboration of the systems of biological protection and the development of

diagnostic, medicinal, and prophylactic means and methods.

## SPECIATION OF MICROORGANISMS: GENERAL REMARKS

The study of the factors and regularities of the origin and evolution of pathogenic microorganisms is a prerogative of evolutionary theory, as in the case of all living organisms. At present, the synthetic theory of evolution (STE) is being developed successfully and is integrating noncontradictory propositions, principles, and facts borrowed from other, so-called non-Darwinian evolutionary concepts (Kolchinskii, 2002). At the same time, non-Darwinian concepts continue to exist and accumulate new arguments and facts. The opposition to the propositions of STE is traced well enough on the part of microbiology and molecular genetics (MG) of microorganisms (Zavarzin, 1974, 1990, 1993). In the problems of the origin of new species and intraspecies forms of pathogenic microorganisms, on the one hand, MG is thought to be the front line of the biological science absolutely dominating over classic directions; on the other hand, it is characterized by obvious methodological reductionism devoid of the biological content and ignoring the adaptation approach.

Such one-track striving for absolute leadership in the problems of evolution is likely to be a temporary and transient phenomenon. MG is a young, promis-

ing, vigorously developing, and ambitious field of biological science, but it is still far from self-sufficient in evolutionary aspects. At present, molecular regularities of evolution can hardly be traced (Pavlinov, 2005a, 2005b).

The conclusions of molecular genetics about the origin of new species of the causative agents of diseases and intraspecies forms have been made taking into account variability and the results of the comparative analysis of convenient molecular marker structures; as a rule, without reliable substantiation of their homology. The consequences of molecular restructuring of genomes in different living organisms, from microbes to higher animals and plants, often cannot be interpreted satisfactorily from the position of functionality and adaptation (Pavlinov, 2005a, 2005b; Abramson, 2007). Microbial clusters revealed with MG methods for individual or several markers sometimes differ significantly among each other and frequently do not correspond to the clusters identified by ecological, biogeographical, and biochemical characteristics. As early as fifty years ago, Mayr (1963, p. 432) wrote: "Species differences cannot be expressed in genetic units of information, i.e., in nucleotide pairs... The integration level that makes sense is much higher than the level of the main informational code, i.e., nucleotide pairs." Speciation is, in larger measure, the subject of study of population genetics (hereditary variability), ecology (struggle for existence), biogeocenology (coadaptive complexes, ecological niches), and other supramolecular sciences, i.e., the subject of study of the formation of epigenetic systems in a changeable environment.

The beginning of the development of any specific hypothesis about the origin and evolution of some species should be preceded by a sufficiently general evolutionary scenario (Pavlinov, 2005b). The algorithms of plausible phylogenetic reconstructions are developed on its basis. We cannot devise phylogenetic schemes using only new and promising, but highly specific MG-methods and findings, which is the usual practice at present. When we study the origin and evolution of any species, what is needed is consolidation of sciences that study life at different levels of its organization, from molecular to biogeocenotic. For this purpose, convenient adequate models are required. The ecological scenario of the origin and evolution of the plague pathogen (the microbe *Yersinia pestis*) is one such model.

We dwell on the correspondence of some facts and regularities presented in our ecological scenario of the origin and evolution of the plague pathogen to the main propositions of the STE. This will enable us to provide convincing arguments in favor of the necessity of using (in combination with the molecular data) the classical procedure to the study of the origin and evolution of the plague pathogen and construction of phylograms. The suitability of the ecological scenario to the propositions of STE must become a new stimulus

for such a necessary integration of indisputable achievements of molecular genetics of microorganisms into classic propositions of modern evolutionary theory.

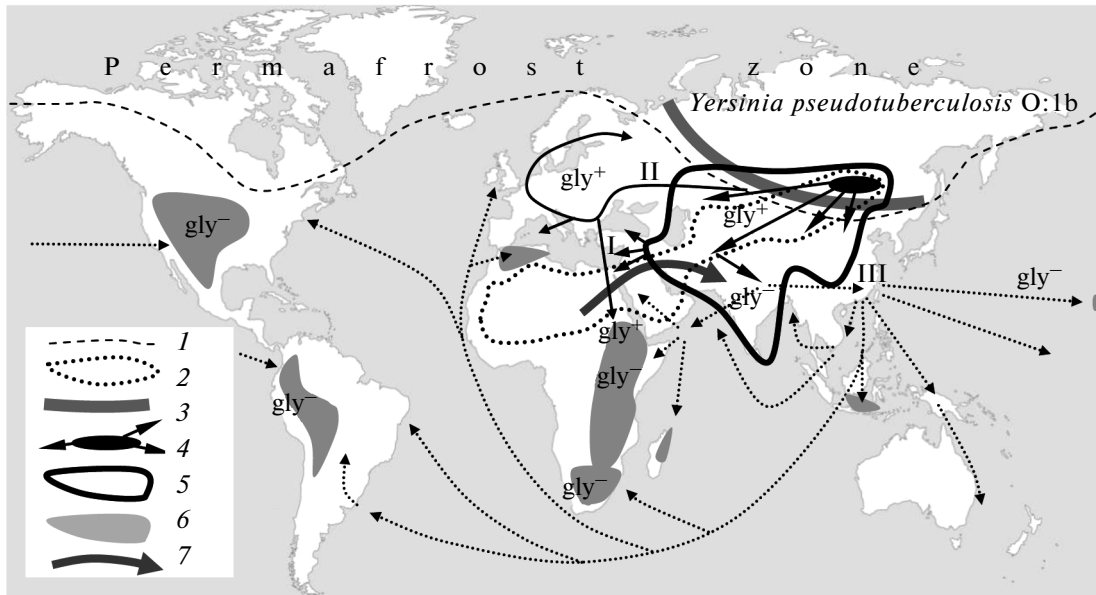
#### MG-SCENARIOS OF THE ORIGIN OF THE PLAGUE MICROBE

At present, the MG-investigators of microorganisms are being primarily guided by the saltation principles of the evolution of prokaryotes. This principle has found application in the framework of the evolutionary doctrine of neocatastrophism (Kolchinskii, 2002). The most popular MG-scenarios of the origin of the plague microbe proclaim its rapid, as a result of few molecular genetic acts, separation from the pseudotuberculosis microbe *Yersinia pseudotuberculosis* in the populations of small homoeothermic mammals—Microtinae voles—no more than 20000 years ago (Achtman et al., 1999, 2004; Morelli et al., 2010; Achtman, 2012; Sun et al., 2014). Different authors refer to the Caucasus, the Middle Asia mountains, China, and the African continent as the places where the microbe originated. The speciation process is linked to horizontal transfer and insertion of foreign genes into the genome of the initial pseudotuberculosis microbe, as well as the inactivation and the loss of genes in a new host. Two acquired plasmids, *pPst* and *pFra*, specific to the plague microbe (it is not clear why specifically if they were acquired from the outside), are supposed to play the most essential role.

It was established that these plasmids encoded the synthesis of pesticin, the capsule antigen F1, and other proteins responsible for the most important adaptive characteristics of the plague microbe (Zhou et al., 2009; Sun et al., 2014). However, such a saltation approach runs counter to the STE propositions. Single insertions of large foreign genetic structures are not adaptive. Macromutants are not viable in the natural environment due to disorders of morphophysiological correlations and coordination of organs (organoids, organelles) and whole organisms. In the evolutionary MG-studies, owing to their saltation ideology, the task of revealing the mechanisms of gradual morphogenesis of plasmids in a microbial cell and smooth transition of the ancestral population to a new ecological niche (and adaptive zone) is not set; the forms of speciation and the evolutionary factors are not discussed.

#### ECOLOGICAL SCENARIO OF THE ORIGIN AND WORLD EXPANSION OF THE CAUSATIVE AGENT OF PLAGUE

The plague microbe has a number of undeniable advantages over other microbial species as a model subject of the study of the properties of high virulence and pathogenicity of microorganisms. Firstly, its direct ancestor, serotype I pseudotuberculosis microbe *Y. pseudotuberculosis* O:1b (Skurnik et al., 2000), also



**Fig. 1.** Scenario of the origin, natural distribution in Eurasia, and anthropogenic worldwide expansion of the plague microbe *Yersinia pestis* during the first (I), second (II), and third (III) pandemics. (1) The southern boundary of the permafrost zone; (2) the Sahara–Gobi arid zone; (3) the boundary of the dominant prevalence of *Y. pseudotuberculosis* O:1b in nature; (4) the geographic range of the tarbagan marmot *Marmota sibirica*, the region of the origin of the plague microbe and direction of its natural expansion in Eurasia; (5) the geographic range of primary natural foci; (6) the geographic ranges of secondary natural foci; (7) propagation of a representative of the genus *Tatera* from tropical Africa to the Asian continent in the Early Pleistocene. gly<sup>+</sup> (gly<sup>-</sup>) is the strain ability (inability) to ferment glycerin. Dotted arrows show the pathways of anthropogenic propagation of the subspecies possessing the gly<sup>-</sup> feature from the Hindustan populations of *T. indica*.

widespread in the world in our time, has been revealed. Secondly, MG-methods reliably substantiated the time of isolation of the plague microbe from the population of the pseudotuberculosis microbe—the Late Pleistocene–Holocene era (Achtman et al., 1999, 2004). Thirdly, there is necessary and quite sufficient evidence of its marmot *antiqua* form revealing some characteristics of genuine antiquity and its habitat; in particular, this form (biovar) circulates in the host-parasite Mongolian tarbagan marmot (*Marmota sibirica*)–marmot flea *Oropsylla silantiewi* system. Fourthly, historic biology and paleoclimatology have accumulated extensive factual material and theoretical knowledge concerning the history of biotas, biogeocenoses, biomes, and the Earth’s climate in the Late Pleistocene and the Holocene. This creates a reliable scientific basis for reconstruction of the evolutionary events of the Quaternary and, thus, for a reliable calibration of the “molecular clock” of the plague pathogen.

The ecological scenario of the origin and evolution of the plague microbe proposed by us earlier (Suntsov and Suntsova, 2000) differs strikingly from the multitude of extremely reduced phylogenetic MG-constructions (Zhou et al., 2004; Achtman, 2008, 2012; Kutnyrev et al., 2009; Morelli et al., 2010; Sun et al., 2014) in the more rich biological content, the possibility of testing and verifying conclusions and factors, and the perception of the reality and interaction in

time and space of the evolutionary events described. We cite its main theses (Fig. 1).

MG-methods reliably show that the psychrophilic saprozoontic microbe *Y. pseudotuberculosis* of the first serotype is a direct ancestor of the causative agent of plague (Skurnirk et al., 2000). This slightly pathogenic enteric microbe causes a human disease referred to as Far Eastern scarlatiniform (scarlatinoid) fever or a “refrigerator disease” (Somov et al., 2001). Serotype I of the pseudotuberculosis microbe is most widely spread in the environmental organic matter (animal excrements) in the cold regions of Siberia, Central Asia, and Far East (Fukushima et al., 1998, 2001).

According to the molecular clock, the plague microbe separated from the ancestral pseudotuberculosis clone at the end of the Pleistocene–Holocene (Achtman et al., 1999, 2004). Diverse evolutionary events in the northern and central regions of Asia, including the origin of the plague microbe, are connected with the onset at the end of the Late Pleistocene, 22000–15000 years ago (Sartan time), of maximal cooling that caused biocenotic crisis and reshaping of northern ecosystems.

The medium where the speciation process occurred is the parasite Mongolian tarbagan marmot (*Marmota sibirica*)–specific Eurasian marmot flea *Oropsylla silantiewi* system (Suntsov and Suntsova, 2000). The fellow members of this parasite system

have unique ecological (ethological) features, which became a prerequisite for the speciation process.

*Wintering hole plug.* The aridity of the climate determines the low ground humidity in Central Asia, which accounts for 2–7% in the mountain steppe habitats of the tarbagan marmot. Habitation in stations with dry rubble ground determined the difficulties of making a plug in the wintering hole from loose material. Habitation of the Mongolian marmot under the dry climate conditions led to the formation of a species-specific behavior manifested by using its own metabolic water. In contrast to other species, it makes the wintering plug from a specially prepared mixture of fine earth, rubble, vegetable waste, and its own wet fecal masses accumulated in the period of activity in special toilet chambers. Making a wintering plug in the hole before going into hibernation, marmots use their teeth to carry the stones rolled in the excrements. In the process, the fecal particles and the pseudotuberculosis pathogen gain entrance into the oral cavity of a hibernating marmot.

*Familial-colonial lifestyle and hibernation.* Marmots live in families consisting of two to twenty-two animals. During hibernation, marmots wake up 11–15 times. More prolonged periods of deep sleep (torpor) alternate with short (3–6 days) periods of wakefulness (euthermia) (Arnold, 1988, 1993). In the torpor phase, the body temperature is 2–5°C; in the euthermal phase, it may attain normal values noted in active marmots (~37°C). Thus, during the period of underground life in the cold time of the year, every animal has two thermostatic states with an intervening physiological heterothermia intervals.

*Facultative hematophagy of *O. silantiewi* larvae.* Flea larvae are detritophages; cases of parasitism are extremely rare (Vashchenok, 1988). But facultative larval parasitism of *O. silantiewi* is a common phenomenon (Zhovtyi and Peshkov, 1958). The main cause for the larvae to switch over to parasitism is deep freezing of the ground due to a sharply continental climate and severe winters with little snow. The parasitism of *O. silantiewi* larvae is determined by the simplest behavioral reaction—thermotaxis.

Deep freezing of the ground in the winter to the level of the location of wintering locules in the holes of Mongolian marmot (2 m and deeper) induced the speciation of the plague microbe. The temperature decrease in the hibernating area below 0°C caused labile behavioral adaptation of *O. silantiewi* larvae, i.e., transition, by virtue of positive thermotaxis, from cold lining of nest onto the bodies of sleeping marmots. This, in turn, caused facultative hematophagy: the flea larvae migrating in the hairs of hibernating marmots ended up with stochastic regularity in the oral cavity and began to feed on the mucous membrane. Larval scarifications interfering with the integrity of mouth mucosa triggered persistent blood infection—traumatic (mechanical) penetration of the pseudotuberculosis microbe from the fecal particles that entered

the oral cavity during the plug-making activity into the bloodstream. The entrance of the pseudotuberculosis microbe into the bloodstream of a hibernating marmot is an obviously inadaptive, disharmonic process when living blood is mixed with excrements. It is this stable inadaptive process in biocenosis that caused adaptive shifts in the isolated microbial population (clone) and became the key ecological factor of speciation of the plague microbe.

Upon completion of the speciation process in the settlements of the tarbagan marmot, the plague pathogen underwent a wide geographical expansion in Eurasia due to interspecies parasitic contacts of hole-dwelling mammals following the oil spot principle (Fig. 1). On the Hindustan Peninsula, where the plague microbe circulates in the populations of the Indian gerbil *Tatera indica*, the latter lost the ability to ferment glicerol. This is determined by the Afrotropic origin of *Tatera* gerbils, which do not require energy reserves in the form of subcutaneous, intramuscular, and cavital fat. On the contrary, in warm-blooded hosts inhabiting the temperate zone, it is an important reagent of metabolic processes: fat is broken down to fatty acids and glicerol, and the plague-afflicted microbial cell depends on glicerol for its metabolism manifesting the gly<sup>+</sup> trait (Domaradskii, 1998). Glicerol is also a cryoprotector in the body of a large number of species of insects (supposedly, the marmot fleas as well) living under low temperatures conditions.

Anthropogenic factors played the main role in the propagation of plague to the African continent and the New World.

#### SYMPATRIC SPECIATION OF *Y. pestis*

In the STE, the principles, forms, and ways of speciation are actively discussed. Gradualism, a gradual, but not necessarily uniform, species formation is a recognized principle of speciation (the alternatives are catastrophism, saltation). In gradual speciation, geographic (allopatric, spatial) and sympatric (ecological, physiological, genetic) speciation is distinguished (Mayr, 1963). Geographical speciation provides for spatial separation of populations, and sympatric speciation does not. It is customary to consider that in geographical speciation, primary separation of populations is determined by external causes in relation to them; in sympatric speciation, it is determined by internal factors. The wide distribution of geographical speciation in nature is not doubted, while sympatric speciation is the subject of heated discussions.

One of the disputable speciation modes is transition of a population to a new, already existing ecological niche. Note that the initial form must lose the equilibrium adaptive niche state for some time. Wright, a well-known participant in modern evolutionary synthesis (1990), believed that any speciation process has an inadaptive phase and proposed the met-

aphor of adaptive peaks for illustration. A similar metaphor of adaptive lunules was proposed by Shram (1983). Transition to a new ecological niche is accompanied by the population leaving the previous ancestral niche (rolling down from the adaptive peak or rolling out of the adaptive holes) and being for some time on the brink of extinction in an unstable state (Simpson, 1944; Mayr, 1963; Grant, 1991; Rasnitsyn, 2002). According to the definition, one niche is inhabited by only one species, and the niche of the new species must be free to be utilized. Weak competition makes possible quick unilateral specialization: the ancestral species ending up in a depleted biocenosis appears under conditions that are mitigated by many parameters and allow for a quicker and easier transformation of structures and functions (Schwarz, 1980; Krasilov, 1986).

The orthodox scientists adhere to the views described above. At the same time, there are few of those who doubt that, in the final analysis, evolution is always adaptive. That is why discussions about the adaptive and inadaptive components in speciation processes continue up to now. Fundamental ecological niches of species usually consist of narrower subunits characterizing the slightly differing conditions of existence of intraspecies forms (subspecies) within the boundaries of the geographic range of the species. Apart from the characteristic species-inhabited niches, which he called normal, Mayr (1963) also drew attention to the existence of special niches (or subniches), which are rare, exclusive, and marginal, with conditions markedly differing from those of normal niches. It is the lateral conditions on the boundaries of the geographic range of a species to which the author linked the cases of speciation by transition of a population to a new ecological niche.

Let us comment on our scenario of the origin of the plague microbe in light of the STE theses set forth above. According to the scenario, the process of separation of the clone of the plague microbe from the population (clone) of the pseudotuberculosis microbe occurred in the population of the tarbagan marmot and the fleas parasitizing on it outside any geographical isolation; i.e., speciation should indisputably be recognized as sympatric (Fig. 2).

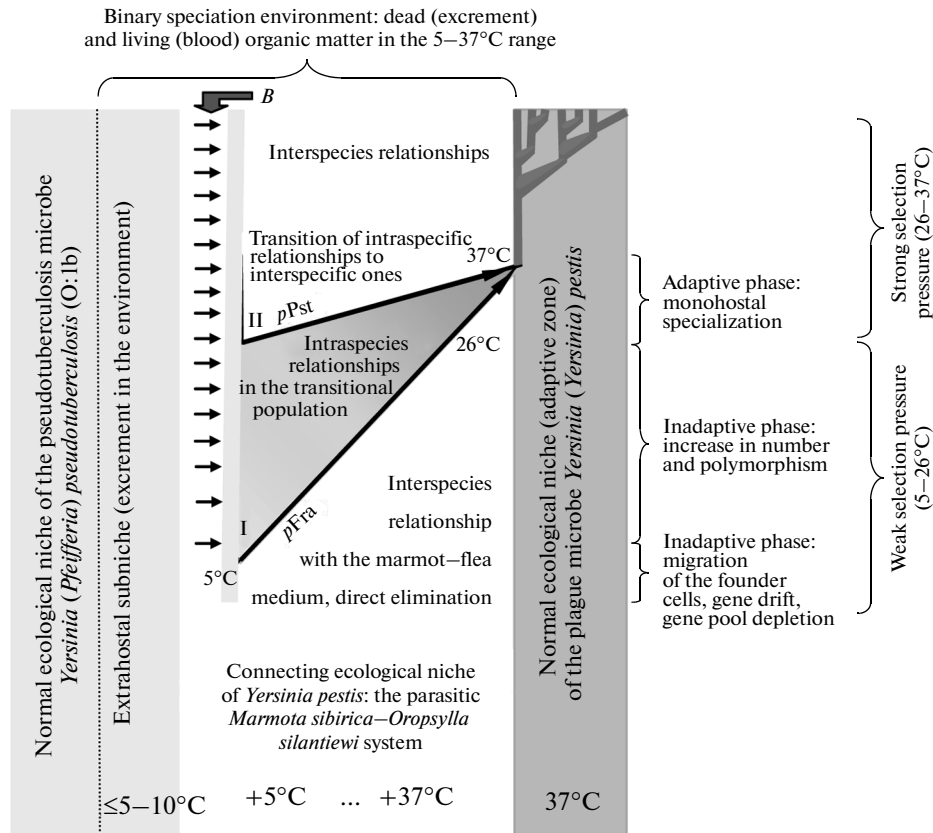
The starting environment for the transitional microbial population was the organic substrate in the external "cold" ( $\leq 5-10^{\circ}\text{C}$ ) medium in the form of an extrahostal subniche of the pseudotuberculosis microbe. The final medium, i.e., the normal ecological niche (hostal subniche) of the plague microbe providing for a continuous epizootic process in the rodent–flea–rodent chain, became the body of warm-blooded ( $37^{\circ}\text{C}$ ) host mammals. The transition between normal ecological niches was realized as a result of two events. The microbe first had to overcome a spatial gap, i.e., to gain entrance from the excrement (the material of the wintering hole plug) into the marmot's oral cavity and then to penetrate the mucous

membrane in the mouth. The former event occurred due to the global transformations of the biosphere during the Cenozoic era that led to aridity of the climate of the Asian continent and, in the final analysis, to the species-specific hole-building behavior of the Mongolian marmot. The second event was initiated by the regional Late Quaternary Sartan cooling, which was maximal in Northern and Central Asia. What occurred as a result of this severe cooling was a shift in the behavior of one of the components of the Central Asian biocenosis: the larvae of the marmot flea *O. silantiewi* began to display the property of hemophagy in the facultative mode. Thus, the barrier between the media of the ancestral and daughter forms of the evolving microbe was overcome under conditions of a unique constellation of biogeocenotic (abiotic and biotic) factors.

After overcoming the biotopic barrier, the pseudotuberculosis microbe had to become anchored in a new medium—the blood of a tarbagan marmot. This became possible owing to its complex (morphological, physiological, biochemical, genetic) preadaptation to habitation in the mammalian lymphomyeloid complex consisting in the presence in the saprozoontic pseudotuberculosis microbe of two isoenzyme systems, which allow habitation in external organic matter and in the animal body (Somov et al., 2001).

The STE does not recognize viable macromutations and, accordingly, puts in doubt the reality of the phenomenon referred to as horizontal gene transfer, which molecular genetics consider as a key process in the speciation process of *Y. pestis*. According to STE, adaptatiogenesis is carried out gradually. As applied to the plague microbe, it follows that there existed a certain adaptive transitional medium between the normal ecological niches of the pseudotuberculosis and plague microbes. Our scenario shows that the heterothermic host-parasite tarbagan marmot–*O. silantiewi* flea system was such a medium. This host-parasitic system forms a unique linker, a connecting ecological niche with a temperature continuum of  $5-37^{\circ}\text{C}$ . All gradual speciation population-genetic and molecular-genetic processes occurred in this unique connecting niche (Fig. 2).

Our plague microbe speciation model shows how the inadaptive aspect of the speciation process should be understood. Sympatric speciation of the plague microbe includes three steps (phases, stages): a decrease in genetic diversity at the expense of gene drift in small groups of migrant founders; an increase in genetic polymorphism with a growing number of the grouping after its having been anchored in a new hostal medium; decreased polymorphism, balancing of the gene pool in the process of specialization in the body of marmots that are active in the warm season and have a constant temperature of  $\sim 37^{\circ}\text{C}$ , and the formation of the initial subspecies *Y. pestis tarbagani* (Suntsov, 2012, 2014a). Speciation at the initial stage is always a risky process. Depletion of the gene pool



**Fig. 2.** Sympatric speciation of the plague microbe: the ratio of the ecological niches of the ancestral and daughter species. I: the ectogenesis vector  $5 \rightarrow 37^\circ\text{C}$  (adaptation of the grouping to the monohostal-vector environment; morphogenesis of plasmid  $p\text{Fra}$ ). II: the autogenesis vector  $26 \rightarrow 37^\circ\text{C}$  (formation of the initial population of *Yersinia pestis tarbagani*; morphogenesis of plasmid  $p\text{Pst}$ ). *B* denotes “a unilateral ecological barrier between two environments.”

due to gene drift and the associated genetic instability lead to death rather than to success (Mayr, 1963). At the second stage, the uncontrolled swelling of the gene pool of the populations (groupings, clones) established in a new medium also leads to their genetic misbalancing and instability. According to the STE positions, the first two steps can be characterized as inadaptive phases when accidental events dominate. Only at the third stage, in the adaptive phase, do the intraspecies relationships begin to be regulated by pest-tic; selection proceeds most efficiently and results in the formation of the initial population, which is true persistent (Fig. 2). Adaptation to the body of an active marmot ( $37^\circ\text{C}$ ) allowed the plague microbe to occupy a “normal” niche and to embrace the populations of constantly warm-blooded hosts: gerbils, voles, pikas, rats, and other animals.

Two factors are of importance in the utilization of any new ecological niche: competitors and predators (Mayr, 1963). In our scenario, the blood of healthy, robust mammals is an absolutely sterile medium, so the influence of competitors can be neglected. The role of a predator for a prospective plague microbe was played by macrophage phagocytes (Klein et al., 2012;

Pradel et al., 2014). It is the interaction with macrophages that initiated the process of adaptatiogenesis to habitation in the *M. sibirica*–*O. silantiewi* system resulting in the formation of the mechanism of the microbial resistance to the cell-mediated immunity of the tarbagan marmot and the appearance of the phenomenon of incomplete phagocytosis affording further persistence of the microbe in the body of the host marmot.

It is seen that the scenario of the origin of the plague microbe corresponds well to the above-mentioned STE theses. Owing to the rarest physico-geographical and ecological prerequisites established at the end of the Pleistocene era in the Central Asian mountain steppe biogeocenosis, to be more exact, in the parasite tarbagan marmot–*O. silantiewi* flea system, and in the presence of the necessary preadaptations in the microbe itself, the initial clone of the pseudotuberculosis microbe appeared under the canalized conditions with a selection vector of  $5 \rightarrow 37^\circ\text{C}$  to finally occupy a new ecological niche and even a new adaptive zone.

## TWO STEPS OF ISOLATION

A species is a protected gene pool (Mayr, 1970). The mechanisms that isolate a species are an important species-inherent characteristic. There exists a special system of special adjustments supporting a hiatus between species. The reliability of gaps is always provided for by several, instead of one, mechanisms working simultaneously or in a certain sequence (Grant, 1991).

Two isolation forms were involved in the formation of the plague pathogen: spatial–ecological (biotopic) followed by biochemical (genetic, physiological) (Suntsov, 2015). The function of the former isolation mechanism consisted in creating conditions for transformation of the gene pool under new conditions; of the latter, in preventing a new genetic system from being destroyed in a new ecological niche. Actually, these two forms of isolation correspond to the spatial (prepopulation) and reproductive (postcopulation) isolations described in STE for all living higher animals. The spatial (biotopic, ecological) isolation of the clone of the pseudotuberculosis microbe that gained entrance into the cold blood of a hibernating tarbagan marmot from the excrement of the hole plug is evident. Obviously, sympatric speciation of the plague microbe began with spatial dissociation. Thus, our scenario gives evidence in favor of Mayr's idea (1963) of the priority of spatial dissociation in any speciation. In our scenario, such dissociation occurred in a dynamic finely mosaic scintillating medium represented by the population of heterothermic and heteroimmune tarbagan marmots and the fleas parasitizing on them in the cold period of the year.

Spatial–ecological isolation was a prelude for the emergence of the species *Y. pestis*. At first, it created prerequisites for gene drift and then conditions for the accumulation of mutations and the formation of a 'mobilization reserve of genetic variability' (Schmalhausen, 1968). For the originating species to be able to complete the speciation process, it must acquire sufficient differences in relation to the use of the previous ecological niche. In the plague microbe, such differences occurred due to the appearance of the apomorphic characteristic, namely, the synthesis of the bacteriocin pesticin. Bacteriocins are biologically active proteins with bactericidal properties possessing a comparatively narrow spectrum of actions. As a rule, they are toxic to closely related bacteria identified with the same species but not to conspecific species and the closest clones (Riley and Wertz, 2002; Riley, 2011). Pesticin is a bacteriocin specific to the plague microbe whose synthesis is encoded by the small plasmid *pPst*. Pesticin actively inhibits the growth of the ancestral serotype I pseudotuberculosis microbe. It is pesticin that created interspecies the hiatus. Due to the evolutionary youth of the plague pathogen, the process of its separation from the ancestral form has not reached the peak; therefore, the causative agents of plague and

pseudotuberculosis still remain ecological antagonists and cannot coexist under sympatric conditions in the body of one animal; the plague microbe is always the winner (Martinevskii, 1969; Aparin and Golubinskii, 1989).

Thus, as proclaimed by the STE, two different mechanisms of isolation were involved in sympatric speciation. The first mechanism is biocenotic, external, which mechanically separated from the population the grouping of migrant founders that took the path of speciation. The second mechanism is an intrapopulation mechanism. This biochemical (physiological, genetic) mechanism separated the initial and daughter forms at the genetic level and guaranteed preservation of the species isolation of the latter.

## STRUGGLE FOR EXISTENCE

The study of the struggle for existence as the main factor of evolution is the prerogative of ecology. As applied to the plague microbe, no special attention was given earlier to the struggle for existence. But quite a large number of basic facts in relation to the plague and pseudotuberculosis pathogens have been accumulated so far to outline the place of this primary factor of evolution in the origin of the plague microbe. Thus, what was the essence of the struggle for existence in the process of evolutionary formation of the plague microbe?

Earlier, we noted that the adaptiogenesis of the plague microbe in the intermediate tarbagan marmot–*O. silantiewi* flea medium includes two vectors: ectogenesis and autogenesis (Fig. 2) (Suntsov, 2014a, 2014b). Ectogenesis reflects the essence of interspecies struggle for existence; autogenesis, intraspecies struggle.

The resistance of the would-be plague cell to the factors of cell-mediated immunity of the tarbagan marmot (tolerance of phagocytosis, persistence in macrophages, entrance into the extracellular medium), adaptation to persistence in the extracellular medium (blood serum), and the formation of the mechanisms of resistance to the host factors of humoral immunity, as well as adaptation to the flea body (creation of a biofilm, realization of block transfer of the microbe to a new host during the season of terrestrial activity of marmots), were formed in the process of ectogenesis (Hinnebusch, 2005; Eisen and Gage, 2009; Sun et al., 2014). So, the main result of ectogenesis is inactivation of macrophages by the plague microbe, the appearance of the phenomenon of incomplete phagocytosis and block transmission by the fleas.

The process of autogenesis includes the following sequential population and genetic events: migration of scarce pseudotuberculosis cells from the fecal components of the wintering plug into the cold blood of a hibernating tarbagan marmot, gene drift, further increase in the number of the microbial mass in the cold blood of the tarbagan marmot, expansion of non-

structural (diffuse, continuous) polymorphism, pesti-  
cin synthesis, the emergence of intrapopulation  
genetic antagonism and hiatus formation, monohostal  
specialization, and stabilization of traits (Suntsov,  
2012). The final result of autogenesis is a true popula-  
tion of the microbe *Y. pestis tarbagani* possessing the  
properties of self-organization, self-regulation, and  
self-reproduction.

Thus, the scenario of the origin of the plague  
microbe demonstrates that the struggle for existence  
on two fronts—the interspecies and intraspecies  
ones—never ceases, and the complex process of adap-  
tatiogenesis is never interrupted during transition to a  
new free ecological niche.

### NATURAL SELECTION

According to the STE, any evolutionary process  
includes two stages (Dubinin, 1966; Schmalhausen,  
1968). The first stage consists in the creation of geno-  
typic variability. At this stage, accidental processes  
prevail; the selection pressure is weak. The second  
stage consists in the selection of the genotypes that will  
produce the next generation. At this stage, natural  
selection plays a leading role. Mayr (1963) character-  
ized these stages as follows: “Randomness creates dis-  
order; selection creates orderliness. Randomness dis-  
orients; selection directs. Randomness is often  
destructive; selection is often creative. However, both  
randomness and selection are statistical phenomena,  
and this enables them not only to coexist but also, it  
can be said, to cooperate harmoniously.” Thus, one  
can see two components of a single evolutionary pro-  
cess—inadaptive and adaptive—in randomness and  
selection. Let us relate our scenario of the origin of the  
plague microbe to this thesis.

The ecological scenario proposed by us is relatively  
simple and can be analyzed in detail, because it has an  
obviously unified, integral selection vector—temper-  
ature, namely, the body temperature of the Mongolian  
marmot in the physiological euthermia–torpor–euth-  
ermia (37–5–37°C) cycle. The heterothermic body of  
the tarbagan marmot and the poikilothermic body of  
the flea were an external environment for the would-be  
plague microbe in the process of its formation as a new  
species. The rate of immune (cellular and humoral)  
processes in the host and vector bodies depends on  
their body temperatures. Therefore, the whole gamut  
of adjustments of the clone of the cold-loving  
saprophytic (saprozoonotic) microbe, which took the  
path of adaptation to habitation in the body of  
homoeothermic host mammals and vector fleas, can  
be reduced to temperature-canalized adaptation by  
the 5 → 37°C vector (deeply hibernating → active  
marmot) (Fig. 2).

As noted above, the structure of the speciation pro-  
cess includes the following sequential steps (stages,  
phases): gene drift under bottleneck conditions in  
accidental penetration of pseudotuberculosis cells into

the bloodstream of a hibernating marmot; an increase  
in the microbe population in the population of hetero-  
thermic marmots; specialization, stabilization of the  
traits; reduction in intrapopulation polymorphism;  
and the emergence of the initial subspecies *Y. pestis  
tarbagani* (Suntsov, 2012). The role of selection at  
these stages is not unambiguous. At the first steps (drift  
and polymorphism increase) accidental events pre-  
dominate, but they prepare the population for strict  
selection by the apomorphic characteristics. At the  
final stage, selection prevails over accidental events.  
Motive selection by the characteristic of increased  
pesti-  
cin synthesis led to the emergence of a new spe-  
cies.

### CONCLUSIONS

The ecological scenario of the origin and evolution  
of the plague microbe presented by us has many  
advantages over others. Two of them are most impor-  
tant. Firstly, this scenario elegantly fits the general pic-  
ture of the development of nature on the Asian conti-  
nent in the late Cenozoic era, in particular, at the  
boundary of the Pleistocene and the Holocene, the  
time when the plague microbe originated (according  
to the MG-data). Secondly, the facts and regularities  
set forth in it fully correspond to the STE propositions  
formulated, sharpened, and tested on canonic models  
and in numerous experiments.

The considerations about the form of speciation,  
the environment in which formation of the plague  
microbe occurred as an independent species, the eval-  
uation of the main factors of evolution— isolation,  
struggle for existence, natural selection—described in  
the ecological scenario are perceived with more trust  
than the phylogenetic MG constructions. That is why  
the ecological scenario should be adopted as a priority,  
as a zero hypothesis. It is the so-called ecological can-  
vas into which the molecular and MG-data should be  
interwoven. This will denote the beginning of the syn-  
thesis of the classic and molecular approaches to the  
problem of the origin of the plague microbe. Only the  
synthesis of classic sciences, modern molecular biol-  
ogy, and molecular genetics will allow us to unravel  
fully the secrets of the emergence of the properties of  
high virulence and high pathogenicity of the plague  
microbe and to find rational ways to fight them.

It remains to be added that the STE has acquired in  
the form of the ecological scenario of the origin and  
evolution of the plague microbe an illustrative evolu-  
tionary model giving evidence in favor of the signifi-  
cance and efficiency of its propositions, regularities,  
and laws, which can be trusted and relied upon when  
we make a decision on urgent theoretical and practical  
problems of modern biological science.



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Translated by E. Babchenko