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Ecological Aspects of the Origin of *Yersinia pestis*, Causative Agent of the Plague: Concept of Intermediate Environment

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Abstract—Modern phylogenies of *Yersinia pestis* (Logh.), causative agent of the plague, constructed using molecular–genetic methods, do not receive a satisfactory functional and adaptive interpretation and are far from being ecologically valid. We have presented an ecological scenario of the origin of the causative agent of the plague through the transition of the initial pseudotuberculosis microbe *Yersinia pseudotuberculosis* O:1b to a free hostal ecological niche (and a new adaptive zone) under ultracontinental climatic conditions of the Late Pleistocene (Sartan time, 22000–15000 years ago) in southern Siberia and Central Asia. An intermediate environment, i.e., the "Mongolian marmot *Marmota sibirica*–flea *Oropsylla silatiewi*" parasitic system, where the process of adaptation development of the plague microbe took place, has been characterized. A scenario based on the major principles of the modern synthetic theory of evolution opens the way to an ecological–genetic synthesis of the problem of plague origin and is an appropriate model for developing a theory of molecular evolution of pathogenic (plaguelike) microorganisms.

Keywords: origin of the causative agent of the plague, Late Pleistocene, *Yersinia pseudotuberculosis*, *Y. pestis*, ecological niche, intermediate environment, *Marmota sibirica*, *Oropsylla silantiewi*

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The enigma of the origin of plague, one of the most odious calamities in human history, has tortured the progressive minds of mankind since Biblical times. The inerasable recollections of the worldwide plague pandemics remained in human memory mainly due to the three most notorious pandemics that happened in the 6th (the plague of Justinian), 14th (the Black Death), and the late 19th century and beginning of the 20th century (Third Pandemic). From the beginning of the last millennium, interest in the origin of the plague has visibly revived due to the acute problem of emerging and reemerging diseases, including the plague, in the transformed anthropogenic environment. However, the greatest concern is that the causative agent of the plague may be used as a biological agent of category A in bacteriological weapons and bioterrorism. Therefore, science faces an urgent problem consisting of revealing the methods of and conditions under which the known pathogenic microorganisms become highly virulent, as well as providing preemptive theoretical support for the development of biosafety systems. The discovery of natural processes that caused the appearance of the causative agent of the plague in situ is a matter of crucial importance.

Over the last one and a half or two decades, the problem of the origin of the plague has become a prerogative of molecular genetics (MG) (Lindler, 2009; Morelli et al., 2010). However, MG is a young science and the molecular patterns of biological evolution have been only partly discovered. The consequences of molecular transformations in genomes of the majority of studied life forms from microbes to higher animals and plants do not, as a rule, receive a sufficient functional and adaptive interpretation (Pavlinov, 2005a, b and Abramson, 2007). Microbe clusters revealed in relation to single or several indices using the MG methods often do not correspond to the clusters revealed based on the ecological and biochemical parameters, whereas the ecological and other "classic" parameters in many cases allow one to evaluate rather positively the role of microbe populations (strains and clones) in ecosystems and to indicate the taxonomic rank (Cohan, 2006).

As for the causative agent of the plague, namely the microbe *Yersinia pestis* (Logh.), the phylogenies constructed by different authors who used various MG indices differ considerably. Over the last 3–5 years, scenarios of the recent origin of plague microbe from the pseudotuberculosis microbe in the populations of *Arvicolinae* species have become quite popular among MG researchers. (Eppinger et al., 2010; Morelli et al., 2010; Achtman, 2012; Bos et al., 2012). *Y. pestis caucasica*, *Y. pestis pestoides*, *Y. pestis microtus*, *Y. pestis angola*, and some other microbes are considered the initial and most ancient subspecies of plague agent. These forms are minor, additional, nonpandemic, and harmless to humans; have low degree of virulence; and are classified as intermediate forms between the

pseudotuberculosis and plague microbes. However, *Arvicolinae* are warm-blooded nonhibernating rodents and have a constant blood temperature of about 37°C, whereas the pseudotuberculosis microbe is soil psychrophile, i.e., a cold-loving microbe. Therefore, to construct ecological mechanisms of the evolutionary transition of microbes to a fundamentally different living environment, one is obliged to declaratively introduce additional factors and conceptions in these scenarios, i.e., horizontal gene transfer from the remote infectious agents within the system, namely, from salmonella; adaptive macro mutagenesis; one-act saltatory speciation; phylogenetic network; the participation of soil invertebrates (amoebae and nematodes) in the plague microbe speciation, and others. At the same time, these scenarios do not cover the well-known biochemical facts, indicating that *Arvicolinae* subspecies (minor and additional) of the plague causative agent are auxotrophs, which are most dependant on the host organism in terms of amino acid synthesis. These highly specialized subspecies (or forms that passed through the "narrow bottleneck" of local conditions in the course of the natural expansion of the plague microbe in Eurasia and the anthropogenic introduction during the pandemics). For this reason it would be more accurate to consider them the youngest hostal subspecies within *Y. pestis* polytypic species, whereas their affinity with respect to several parameters (rhamnose and melibiose fermentation) between themselves and with the pseudotuberculosis microbe should be considered homoplasies (Variability, 2009; Suntsov and Suntsova, 2006).

In this context it is worth mentioning that recent years were marked by the appearance of studies on the phylogeny of microbes of genus *Yersinia*, in which the MG researchers started using the concepts and terms of the evolutionary biology (Keim, Wagner, 2009; Zhou, Yang, 2009; and Achtman, 2012). It becomes increasingly evident that the MG data can be successfully used in studying the origin of the causative agent of the plague only along with the scientific heritage of traditional sciences, namely, the comparative anatomy, morphology, systematics, ecology, population genetics, paleontology, biogeography, and general theory of evolution. The same thing happened back in the day with phenetics and caryology. At the same time, modern Darwinism, which developed after the incorporation of the ideas of I.I. Schmalhausen (1968), who focused on the whole organism into a synthetic theory of evolution (STE), is considered the soundest evolutionary doctrine (Grant, 1991 and Dobzhanskii, 2010).

According to the STE, the local population represents an elementary unit of evolution. Each population is spatially and temporarily structured. Two components are identified with respect to functional criterion, i.e., core (population of survival stations), which represents its constant component, and the unstable periphery (inhabitants of sinks and parcels) providing for the opportunistic expansion of species (Naumov, 1967 and Pulliam, 1988). Depending on the nature of changes in the environment, taxon, and the particular ecological situation, the formation of a new species may depend on either the constant component of population or the periphery mainly formed by migrants (alien species). Classic didactic examples of the STE illustrate the gradual transformation of the integral structured populations of ancestral species into new species during coevolution with the components of biocenosis (Mayr, 1968, 1974; Schmalhausen, 1968; Timofeev-Resovskii et al., 1977; and Dobzhanskii, 2010). Another type of speciation, namely, by migrants (founders, aliens, and invaders) also reflects well-known evolutionary concepts, i.e., the founder principle, quantum speciation, genetic revolution, island effect, population bottleneck, and source sink (Mayr, 1968, 1974; Grant, 1991; Lidicker, 1988; Sokurenko et al., 2006). This particular type of speciation is typical of the @Y. pestis@ plague microbe.

SCENARIO OF ORIGIN AND GLOBAL EXPANSION OF THE CAUSATIVE AGENT OF THE PLAGUE

Based on the new ecological–geographical, MG, and paleoclimatic data collected over the last one and a half or two decades, one has finally succeeded in developing a rather plausible and consistent scenario of the origin and global expansion of the plague microbe. Among these new data, the following crucial facts can be distinguished: (1) the pseudotuberculosis microbe of serotype 1 (*Y. pseudotuberculosis* Pf. O:1b) represents an ancestral form of the causative agent of the plague [Skurnik et al., 2000]; (2) serotype 1 of the pseudotuberculosis microbe prevail in Northern Asia and the Far East in the regions with severe weather conditions (Somov et al., Fukushima et al., 1998, 2001); (3) the separation of the plague microbe from the pseudotuberculosis microbe happened no earlier than 20 000 years ago (Achtman et al., 1999, 2004); (4) throughout the entire Quaternary Period, deep ground freezing (up to 2 m) in Central Asia was identified only in the Sartan time of the Late Pleistocene and during the Holocene, i.e., no earlier than 22000 years ago (Owen et al., 1998); (5) the larvae of fleas parasitizing on the burrowing hibernating mammals (marmots and susliks) are typical detritivores (saprophages) and inhabit the hosting nest substrate year round, but in the ultracontinental winter anticyclone region in Central Asia, during winter months, when the negative temperatures penetrate deep through the ground and reach the nesting chamber, they move to the body of the nest's host and start feeding on skin derivatives, lymph, and blood that appears from skin wounds (scarifications), especially in the axillary region, in the groin, oral mucosa, and around the anus. Thus, the marmot flea larvae practice facultative hematophagy

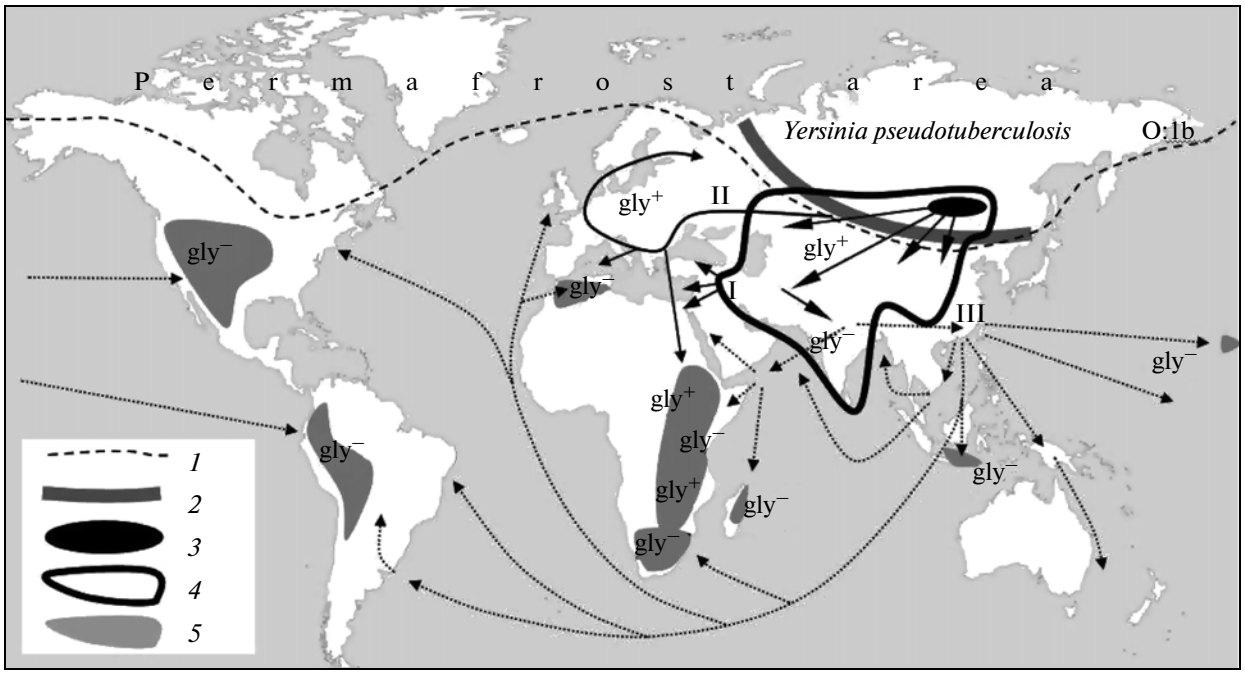

Fig. 1. Scenario of origin, natural expansion in Eurasia, and anthropogenic expansion of the plague microbe *Yersinia pestis* during the first (I), second (II), and third (III) pandemics. (*1*) Southern boundary of the area of permafrost grounds; (*2*) boundary of the dominant distribution of the *Y. pseudotuberculosis* O:1b microbe in nature; (*3*) distribution area of Mongolian marmot, i.e., the area of origin of the plague microbe; (*4*) area of the initial natural foci; (*5*) areas of the secondary natural foci. gly+ (gly$^-$) ability (inability) of strains to ferment glycerin (cryoprotective agent). The gerbil *Tatera incida*—the main host of the causative agent of the plague in the natural foci of Hindustan—and its specific flea *Xenopsylla astia* have Afrotropical origins and cannot adapt physiologically or biochemically to cold conditions (cryoprotectors). The plague agent in their populations possesses a unique gly$^-$ characteristic. From the *T. indica* populations, the subspecies that possessed the gly$^-$ characteristic was distributed anthropogenically all over the world (dotted lines).

during the cold months of the year (Suntsov and Suntsova, 2006).

According to the scenario, the plague causative agent generated in Central Asia during the maximum Quaternary–Sartan cooling in the "Mongolian marmot (*Marmota sibirica* Radde)–flea *Oropsylla silantiewi* Wagn. parasitic system" (Suntsov and Suntsova, 2000, 2006, 2008, 2009, 2010a) (Fig. 1). The other hundreds of natural and anthropogenic parasitic systems "rodent (pika)–flea" known around the world had nothing to do with the origin of the causative agent of the plague. Only a few such systems in which the flea hosts are represented by landscape-forming species that inhabit vast areas and the most widespread and abundant synanthropic rodents were responsible for the natural postglacial distribution of the plague microbe in Eurasia, recent global anthropogenic expansion, and the development of secondary natural foci in Africa, the New World, Java, Madagascar, and Hawaii (Suntsov et al., 2011; Shilova and Suntsov, 2011).

The scenario of the origin of the plague agent is consistent with the evolutionary concept of sympatric speciation through a transition to a new ecological niche and new adaptive zone.

ECOLOGICAL NICHES OF ANCESTRAL AND DAUGHTER SPECIES

Y. pseudotuberculosis and *Y. pestis* microbes are fairly discrete species which normally inhabit fundamentally different environments and occupy different ecological niches and adaptive zones. The notions of ecological niche (fundamental, realized, and potential) and adaptive zone are constructive and useful while considering many issues of the theory of evolution. The fundamental ecological niche ensures the ecological stability of a species; therefore, the speciation through the transition to a new fundamental ecological niche and adaptive zone is accompanied by adaptation that goes beyond the limits of the former niche and zone (Severtsov, 2008).

The fundamental ecological niche of pseudotuberculosis microbe. The pseudotuberculosis causative agent is classified as ubiquitous heterotopic microorganism. It was identified in the alimentary canal (organs) of most diverse animals, i.e., hydrobionts (daphnia and cyclops), benthic invertebrates (annelids, mollusks, and insect larvae), soil amoebae and nematodes, fleas, fish, and many species of wild and domestic birds and mammals. In external (nonorganism and nonhostal) environments, the pseudotuberculosis microbe is mainly located in organic substrates (feces and urine),

where it acquires invasive properties in cold weather conditions with low positive temperatures not exceeding 10–15°C. Inhabiting two different environments, namely, the off-hostal organic environment and the alimentary canal (organs) of animals, the pseudotuberculosis microbe possesses two isoenzyme systems that are activates under appropriate conditions. Its fundamental ecological niche is represented by the life form of the low pathogenic agent of the intestinal saprozoonosis. However, pathogenicity occurs only in relation to animals with an immune system deficiency or exposed to stressful conditions (Somov et al., 2001).

The causative agent of serotype 1 is psychrophilic and intensely reproduces in feces at a temperature of 4–10°C. Compared to other serotypes of the pseudotuberculosis microbe, this agent is phylogenetically younger and more pathogenic for warm-blooded hosts. It may cause the mass mortality of rodents due to the weakening of the immune system and is responsible for human disease known as Far East scarlet-like fever (FESF) (Somov et al., 2001). With respect to several MG indices, this serotype bears the closest resemblance to the plague microbe of the antiqua kind typical of the marmot distribution areas (marmot *Marmota baibacina*–*flea Oropsylla silantiewi*) in southwestern China [Wang et al., 2006]. Of special note is the active reproduction of the pseudotuberculosis microbe in the alimentary canal of fleas exposed to low temperatures of 6–8°C (Vashchenok, 1988). Marmot and suslik fleas, including Mongolian marmot fleas, which inhabit Siberia and Central Asia, are exposed to these and even lower temperatures all year round (Suntsov and Suntsova, 2006).

Fundamental ecological niche of the plague microbe. Under natural conditions, the plague agent parasitizes warm-blooded mammals (Rodentia and Ochotona) and is transmitted by fleas. Thus, the plague microbe, just like its ancestor, namely, the FESF agent, has two different habitats, i.e., the hostal habitat with a temperature of 37°C (body temperature of an active warm-blooded host) and the vector habitat, namely, the body of the poikilothermic flea.

The microbe is localized in the lymphomyeloid complex of the warm-blooded host body (Perry, Fetherston, 1997). The interaction of the plague microbe with the warm-blooded host body is carried out through the immune (cellular and humoral) mechanisms of a host, the central role being played by macrophages (Klein, Bliska, 2009; Klein et al., 2012). The immune system of hibernating mammals depends on the body temperature: during hibernation accompanied by the decline in body temperature, all physiological processes decelerate and the level of the host's immune response to antigenic impact falls drastically.

As for fleas, the plague microbes inhabit only the content of their alimentary canal, i.e., the environment generated by a warm-blooded host, and does not penetrate the tissues of the insect itself. This suggests a relatively weak evolutionary connection between flea and microbe. During each feeding on the invaded host body, the flea normally sucks in tens and hundreds of the single "planktonic" microbe cells, some of which remain for a short time in the flea's piercing mouthparts. In this case, within a short time after feeding of the infected flea on a new host, the transmission of microbes performs very rapidly according to the mechanical (primitive) scenario (Suleimenov, 2004; Vetter et al., 2010). Within longer time frames after bloodsucking (weeks and months), if the infected flea resides in the nest substrate under cold temperatures, the microbes reproduce in its proventriculus and form a biofilm–aggregations of microbes. These aggregations partly or fully fill the lumen of the flea's alimentary canal, creating a bacterial block. The transmission is performed through the "eructation" of the proventriculus content into the bite spot while bloodsucking, that is, through the mechanism of block forming. Such a transmission mechanism developed as a result of closer and longer physiological, biochemical, and coevolutionary interactions between the populations of fleas and their hosts (Bibikova and Klassovskii, 1974; Zhou and Yang, 2011; and Chouikha and Hinnebusch, 2012).

On the whole, with respect to the main characteristics of its interactions with the environment, the plague microbe occupies the fundamental ecological niche (and adaptive zone) of the transmissible facultative intracellular zoonosis agent, the highly pathogenic obligate blood parasite of the burrowing mammals, and both hibernating (marmots, susliks, and prairie dogs) and nonhibernating (gerbils, voles, and pikas, as well as synanthropic rats, bandicoot rats, and house shrews under conditions of anthropogenic impact).

INTERMEDIATE NVIRONMENT BETWEEN THE ECOLOGICAL NICHES OF ANCESTRAL AND DAUGHTER SPECIES

The notion of an intermediate environment is not used when studying the speciation and constructing the phylogenies based on MG methods. Our ecological approach decisively proves that the speciation of the plague microbe occurred through a gradual development of adaptation to a particular two-component intermediate environment at a temperature ranging from 5 to 37°C. The evolution started with the population of the pseudotuberculosis microbe that was found in the feces of Mongolian marmot. The speciation completed with the appearance of a fully functional population capable of stable circulation within the populations of many mammal species with a constant body temperature of 37°C. Moreover, the vacant niche for the plague microbe of the initial subspecies (subniche) *Y. pestis tarbagani* (Mongolian marmot–flea *O. silatiewi* system") arguably existed as early as Pliocene, millions of years before the appearance of the microbe itself. Paleontological findings of marmots (genus *Marmota*) date back to the Oligocene

(Bibikov, 1967), and the Mongolian marmot was identified in the Early Pleistocene (Erbaeva, 1970). Fleas represent a phylogenetically ancient group of ectoparasites of warm-blooded vertebrates. The ancient paleontological findings of this group date back to the Quaternary Period (Eocene–Oligocene) (Vashchenok, 1988). The question arises as to the properties of the environment, which facilitated the persistence of intermediate forms in the "inadaptive" state (compromise and adapted in the short term) between two fundamental ecological niches and accomplished the speciation. Let us consider the crucial characteristics of this intermediate environment.

MONGOLIAN MARMOT AS THE INITIAL HOST OF THE CAUSATIVE AGENT OF THE PLAGUE

The most vital factors that determined the initiative role of the Mongolian marmot in the appearance of the causative agent of the plague was its behavior related to the habit to plug the access holes to its burrow when it hibernates and its physiological traits as a heterothermal hibernating animal living in family colonies.

The plug in the hibernation burrow. An arid climate causes low ground moisture in Central Asia, which reaches 2–7% in the mountain and steppe habitats of the Mongolian marmot (Suntsov and Suntsova, 2006). Stations with dry rubble ground are difficulties when creating a plug in the hibernation burrow. A soil moisture deficit provoked the development of species-typical behavior in Mongolian marmot, which started using its own metabolic water. Unlike other species, it creates hibernation plugs using the intentionally prepared mixture of fine earth, crushed stone, and moist feces accumulated during the active period in special excrement chambers. When the marmots plug the tunnel leading to the burrow preparing for hibernation, they drag stones rolled in feces with their own teeth. The feces particles, along with the pseudotuberculosis agent, land in the mouth cavity of the marmot, which enters a state of torpor. The pseudotuberculosis causative agent has been on multiple occasions identified in marmots while studying the plague foci in Central and Middle Asia (Suntsov and Suntsova, 2006).

Colonial way of life and winter hibernation. Marmots are typical colonial animals. The colonies are made up of family units, i.e., groups of animals that hibernate in one constant or hibernation burrow (Bibikov, 1967). The families are comprised of 2–22 unevenly aged animals of both sexes. During winter hibernation, each animal passes through the bouts of deep torpor that alternate with the shorter periods of sporadic arousal (euthermia) (Bibikov, 1967 and Arnold, 1988, 1993). During topor, the body temperature decreases to 2–5°C, whereas it can reach normal parameters during the euthermic state, namely, 37°C, i.e., the temperature typical of active marmots. During autumn, winter, and spring months, marmots awake not quite synchronically 11 to 15 times, about twice a month (Figs. 2a–2b). Thus, throughout the period of underground life, during cold season, each animal goes through two homeostatic states with an interval of physiological heterothermy between.

The possibility for a flea to feed on an animal with a body temperature ranging from 5 to 37°C is expected to be considerably higher in the complex stable unevenly aged families comprised of a great number of animals and representing the core of populations (Suntsov, 1981).

OROPSYLLA SILANTIEWI FLEA AS THE INITIAL CARRIER OF THE PLAGUE AGENT

The transition to a new specific niche begins almost always from slight physiological changes, in particular, in behavior (Mayr, 1968; Schmalhausen, 1968). The development of the plague agent also started with the changes in behavior, not the behavior of the microbe itself, but that of the marmot flea larvae *O. silantiewi*.

Facultative hematophagy of the *O. silantiewi* larvae. Flea larvae are detritivores and parasitize rather rarely (Vashchenok, 1988). Transitions to warm-blooded hosts, which are genetically fixed in the development cycles, are typical for the flea larvae of nomadic animals and the animals inhabiting the areas with rather severe weather conditions. There have been cases of larvae parasitism, when the general connection with hosts is characterized as a topical one, i.e., parasitism in sick animals and the transition from the burrow to the hibernating animals (Rotschild, 1975). In southern Siberia, *Ceratophyllus tesquorum* and *O. Asiatica* larvae migrate to the long-tailed marmots and the Daurian ground squirrels that hibernate in frozen soil (Pauller, 1980). The indirect trophic links with warm-blooded hosts were positively identified for the larvae of some flea species. These larvae feed on the host skin derivatives or the feces of fully grown fleas, i.e., semi-digested blood of the host, which accumulates in its dense hair coat and nesting chambers (hares, cats, and hibernating rodents) (Vashchenok, 1988; Zhovtyi and Peshkov, 1958; Pauller, 1980; and Rotschild, 1975).

The *O. silantiewi* facultative larvae parasitism in the Mongolian marmot during the cold season is a common phenomenon (Zhovtyi and Peshkov, 1958). The reason for the transition of larvae to parasitism as a mass population phenomenon was the deep ground freezing under the conditions of an extreme continental climate with dry severe winters. The minimal body temperature of the hibernating Mongolian marmot in a state of a deep topor is about 5°C, whereas the ground temperature around the hibernating den at the end of winter and the beginning of spring is less than –3°C. In such conditions, the *O. silantiewi* larvae is driven by thermotaxis transit to a warmer object, namely, the body of the hibernating marmot. During

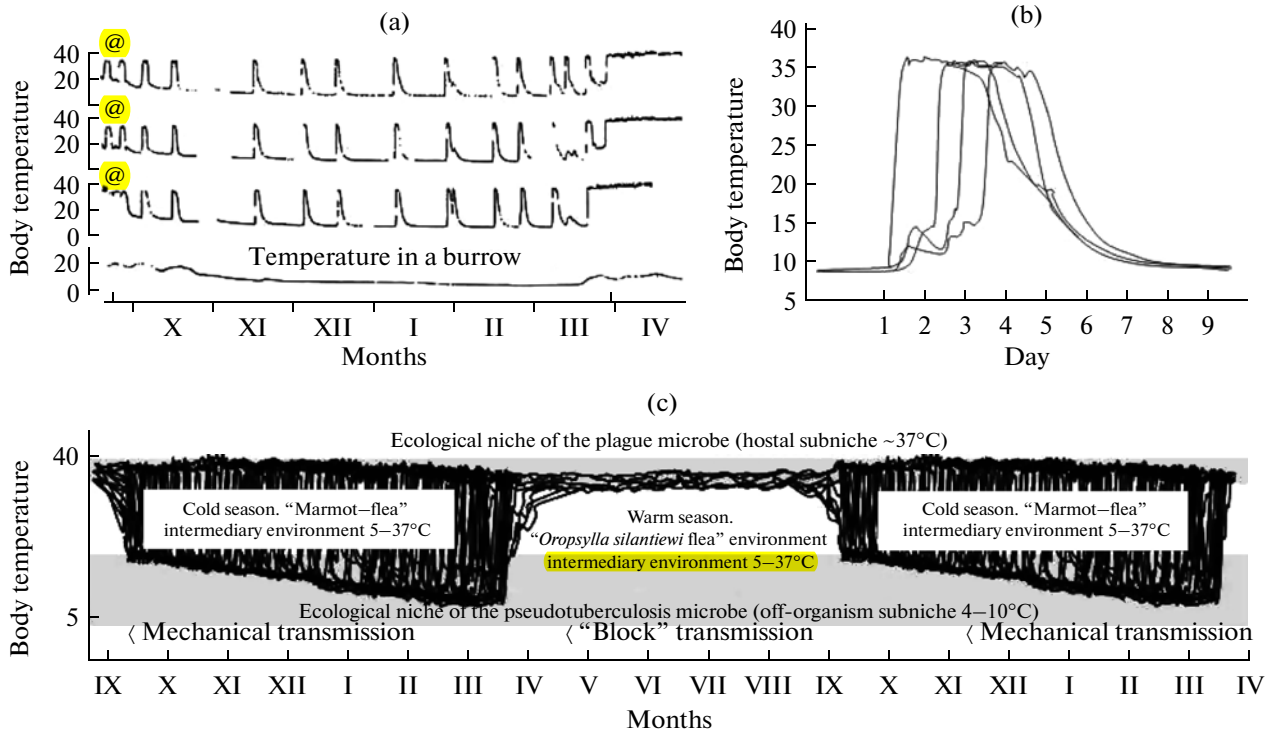

Fig. 2. Dynamics of the body temperature (°C) in marmots during winter hibernation: (A) three Alpine marmots hibernating in group (Arnold, 1993); (B) four animals during a single euthermic period (Arnold, 1988); (C) imitation of the marmots' body temperature dynamics in a group composed of 10–20 unevenly aged animals during autumn and spring months (heterothermal intermediate environment).

such a transition of larvae in the hair coat, some of them lie in the mouth cavity, cut oral mucosa, and feed on the host's lymph and blood appearing from scarifications. The ability of flea larvae to feed on the skin derivatives of warm-blooded animals and the feces of full-grown fleas may be characterized as preadaptation to facultative hematophagy. The emergence of the facultative larvae hematophagy may be with high probability and based on numerous diverse facts dated for the Late Pleistocene. These dates are consistent with the dating of the plague microbe origin based on the MG studies of *Yersinia* (Achtman et al., 1999) and the dating of the permafrost in Central Asia in the Sartan time (22000–15000 years ago) (Owen et al., 1998).

PLAGUE MICROBE SPECIATION AS THE PROCESS OF ADAPTATION DEVELOPMENT IN A HETEROGENEOUS INTERMEDIATE ENVIRONMENT

The process of adaptation development in each population has two aspects that correspond to two forms of a struggle for existence, i.e., interspecific and intraspecific struggles, and includes two inseparable processes, namely, the change in population aimed at protection from the unfavorable abiotic and biotic environmental conditions (ectogenesis) and the transformation that leads a population into a stable state of self-reproduction, self-regulation, and self-organization (Mayr, 1968 and Schmalhausen, 1968). Ectogenesis and autogenesis are followed by morphological adjustments and, consequently, the formation of genetic structures that code evolutionary innovations according to two stated vectors.

Plague microbe ectogenesis. The living environment of every life form and population is always to one extent or another heterogeneous. The heterogeneity of the environment where the speciation of the plague microbe took place consists of its spatial (marmot and flea bodies) and temporary mosaicity (biochemical and immunological variability and temperature pulsation). The temperature continuum of the hostal component of intermediate environment (5–37°C) composed, in our opinion, the essential condition for a gradual evolutionary adaptation of the psychrophilic saprozoon microbe found in the feces, which was used to create a plug in the hibernating burrow of the Mongolian marmot, to the existence in blood (lymphomyeloid complex) of the warm-blooded host with a body temperature of 37°C. The identification of this specific heterothermal (heteroimmune) environment that existed in a particular physical–geographical region opened up a way to develop a gradualistic model of the origin of the plague.

The *O. silantiewi* larvae played an important but simply mechanical role in the evolutionary develop-

ment of the plague microbe by creating the traumatic lesions through scarifications in the mouth cavity, which allowed the pseudotuberculosis microbe to intrude the lymphomyeloid complex of marmot. The larvae ensured the trivial blood contamination on a global all-population scale. The further evolutionary transformation of the microbe was determined only by fully grown animals–carriers. The proportion and the comparative role of each known transmission method, i.e., the mechanical method and that accompanied by the block formation and "eructation," were not studied for the *O. silantiewi* marmot flea. Arguably, the method of a rapid mechanical transmission within a short time after bloodsucking dominated primarily in the case of intrafamily transmission, in large groups of unevenly aged age animals packed together in one nest during the winter months, when all family members have the same probability of being bitten by fleas. No fundamental coadaptation between microbe and fleas is required (Vetter et al., 2010). During summer months, when the marmots are physiologically active, the pseudotuberculosis microbe is completely eliminated from their bodies. During this season, the major role in preservation of the pseudotuberculosis microbe clones that entered the path of transformation into the plague microbe was played by the infected fleas which inhabited the cold host's nests (–8 to +7°C) and were to make the carrier survive during the period of the above-ground activity of marmots (April–September) (Fig. 2). This required the development of closer evolutionary links between microbe and flea and the formation of a more complicated mechanism of prolonged transmission, i.e., the creation of biofilm and blocks, as well as "eructation," along with the adequate formation of genetic structures which would code the performance of the function (Hinnebusch, 2005; Erickson et al., 2007; Vadyvaloo et al., 2010; Zhou and Yang, 2011; and Chouikha and Hinnebusch, 2012).

The future plague microbe underwent the most substantial changes in the marmot body. The major (key) external factor that required crucial changes in the metabolism of the pseudotuberculosis cell was represented by macrophages of the Mongolian marmot lymphomyeloid complex, or, figuratively speaking, "predators," "enemies" of the microbe, which, depending on the season and the physiological state of host in a given moment, have a particular temperature and, consequently, demonstrate a particular biochemical and immunological (phagocytic) activity. The main evolutionary strategy of the plague microbe consisted in counteracting the host's immune defense, which gradually activated during each euthermic cycle. Numerous specific genetic structures developed to ensure the performance of this function (Klein et al., 2012). Among them, one of the most important was a species-specific *p*Fra plasmid responsible for the synthesis of a microbe capsule that protected the microbe from macrophages (Domadarskii, 1998). The initial genetic structure transformed into this plasmid during morphogenesis is yet unknown. It could have been one of two small cryptic plasmids found in the pseudotuberculosis microbe of serotype 1 (Eppinger et al., 2007).

In the presented model, the continual intermediate environment does not have any discrete temperatures. It is neither divided into temperature or immunological subniches nor quantized (Fig. 3). An infected flea may bite a marmot in any intermediate physiological state between topor and euthermia, and the penetration of microbes into the body of a marmot with a temperature of 5 to 37°C is equally probable. The absence of discrete temperature clusters in the intermediate environment suggests an absence in the transitional population of discrete, sporadic forms of polymorphism (subspecies, ecotypes, ecovariants, clones, morphs of the balanced polymorphism, coherent homogeneous groups, etc.) and allows for identifying only two terminal stable evolutionary forms, i.e., the initial form represented by the pseudotuberculosis microbe and the equifinal form, namely, the plague microbe. The first form is called *Y. pseudotuberculosis sibirica* (O:1b) and the second is called *Y. pestis tarbagani*. The population of the initial subspecies generates the population of a new monomorphic monotypic species (Suntsov and Suntsova, 2000, 2006, 2008, 2009, 2010b).

Plague microbe autogenesis. The life of the species is maintained through intraspecific (intrapopulational) interactions directly responsible for reproduction. These interactions were thoroughly and extensively illustrated by the canonical STE models. They develop during autogenesis, which, when a new population is based on "founders," is divided into two stages (phases, steps): (1) genetic destabilization (the loss of genetic variation of founders due to the genetic drift, the increase of population, and the enhancement of sympatric polymorphism owing to neutral mutations under the conditions of a low pressure of natural selection in a new "mild" heterogeneous environment) and (2) subsequent stabilization (the increasing pressure of natural selection, normalization of genetic variability within one direction, fixation of characteristics, and the acquirement of isolating mechanisms) (Dubinin, 1966; Mayr, 1968; Schmalhausen, 1968; and Keim and Wagner, 2009). If the plague microbe originated during the first stage, the founders were an accumulation of chaotically reproducing but steadily increasing in number microbe cells. Exposed to the vector of natural selection 5 → 37°C, the amorphous group of "migrants–founders" gradually transformed into a pseudopopulation (sensu V. N. Beklimishev (1960)) and afterwards, into a genetically "swollen" highly polymorphous population of the virtual transitional form Y. post-*pseudotuberculosis* (Suntsov, 2012). This stage witnessed an increasing heterogeneity, as well as genetic and phenotypic diversity, and the formation, as I.I. Schmalhausen had it (1968), of a

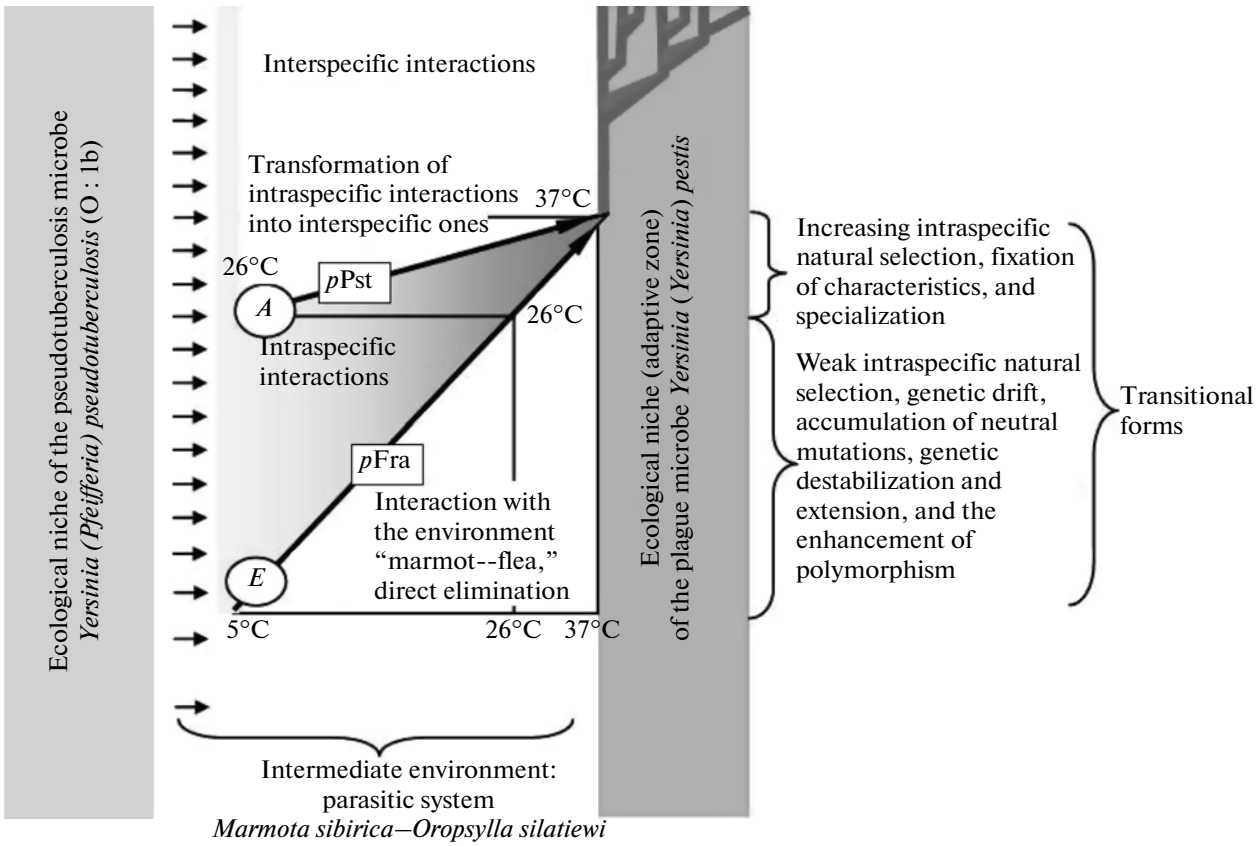

Fig. 3. Gradualistic model of process of adaptation development in the plague microbe: introduction of the saprozoon pseudotuberculosis microbe in a free fundamental ecological niche ("rodent (pika)–flea" parasitic systems) through the intermediate environment *Marmota sibirica–Oropsylla silantiewi*. E–ectogenesis vector of 5–37°C (adaptation to the hostal-vector environment, interaction with macrophages in the host's blood, and the *p*Fra plasmid morphogenesis). (A) autogenesis vector of 26–37°C (intraspecific struggle for existence, emergence of isolating mechanisms, and the *p*Pst plasmid morphogenesis).

"mobilization reserve" of genetic variation, which represented a material basis for rapid speciation.

Continuous (all encompassing, sympatric) polymorphism cannot rise endlessly. There will ultimately be an intrapopulational conflict within a "swollen" population. The first stage of speciation is replaced by the second step, namely, specialization. The transition of the plague microbe to the second autogenesis stage was marked by the synthesis of pesticin bacteriocin, i.e., the appearance of the apomorphic characteristic that "separated" the *Y.* pre-*pestis* new adaptive form resistant to phagocytosis by macrophages from the initial form (Suntsov, 2012). The pesticin synthesis became an intrapopulational physiological (biochemical) mechanism for isolating the *Y.* post-*pseudotuberculosis* and *Y.* pre-*pestis* virtual forms. Hostal specialization was marked by the mutual exclusion of competing genotypes, i.e., antagonistic pleiotropy; exposed to the vector of natural selection 26 → 37°C, the intraspecific interactions gradually transformed into the interspecific interactions between *Y. pseudotuberculosis* and *Y. pestis*. The chromosomal genetic structures and the specific *p*Pst plasmid typical of the plague microbe, as well as the *p*Fra plasmid developed; both specific plasmids became fully operational at the warm-blooded host body temperature of about 37°C.

Thus, the initial subspecies of the *Y. pestis tarbagani* plague microbe developed within the population of Mongolian marmot. Later on (during the Holocene) it was subject to a gradual transformation into a self consistent polytypic species *Yersinia pestis* due to the parasitic contacts during interpopulational and interspecific exchange of fleas between burrowing rodents and pikas in the steppe and mountain steppe landscapes of Eurasia. According to the "oil stain" principle, the mono-, di-, and polihostal natural plague foci developed depending on the structure of particular biocenoses in situ. The number of species of major hosts whose populations suffer from the plague agent in particular foci determines the degree of its hostal specialization (or, vice versa, generalization). Various Arvicolinae populations were covered by the natural expansion of the plague microbe in Eurasia. Thus, the mosaically dispersed Arvicolinae areas and the correspondent Arvicolinae subspecies of causative agent appeared. The natural boundaries of the plague microbe area maintained until the beginning of total nature anthropogenization, which was followed by the

further global expansion of the microbe. Therefore, the conquest of Africa and the New World by the plague was actually done by humans.

CONCLUSIONS

Today there are favorable conditions for studying evolutionary principles and ecological–genetic mechanisms of origin of the causative agent of the plague. A necessary and sufficiently factological base of traditional sciences has been created. There is a reliable theoretical support in the form of the STE. Numerous comparative MG data on the genomic structure of the pseudotuberculosis and plague microbes have been obtained. They are available for interpretation in the context of STE principles.

The identification and description of the intermediate environment between the ecological niches of the pseudotuberculosis and daughter plague microbes opens up wide prospects for developing a theory of molecular evolution of highly pathogenic microorganisms and facilitates the search for the appropriate natural research models, since the populations of both ancestor and daughter species, as well as the environment in which the plague microbe forms (*M. sibirica–O. silantiewi* parasitic system), exist today and are available for performing a comprehensive study, comparative analysis, and experiments.

The proposed ecological scenario of the gradual origin of the causative agent of the plague by the transition into a new ecological niche through intermediate environment represents a clear didactic illustration of the relevance of the STE postulates and a unique model for the further studies on evolution. The scenario outlines the validity borders of molecular and genetic facts, methods, and methodologies concerning the origin and evolution of the plague agent and proves that, provided a correct gradualistic–adaptational interpretation of facts, the MG approaches represent an integral component in exploring the process of speciation. Valid molecular–genetic conclusions enrich classic knowledge on genetic changes in populations that lead to divergence and the development of new species.

The description of the evolutionary principle (gradualism) and the ecological–genetic mechanisms (natural selection in the *M. sibirica–O. silantiewi parasitic system*) of the plague microbe speciation opens up a new way to develop the algorithms and technologies of artificial selection of highly pathogenic and highly virulent plaguelike microorganisms in a man-made gradient temperature–immunological environment and the creation on their basis of a wide range of diagnostic, medical, and preventive anti-infectious preparations.

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SPELL: 1. ekologicheskie, 2. Suntsova, 3. Mezhd, 4. Dang