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# Morphometric, physiological and molecular characteristics of underground populations of the urban mosquito *Culex pipiens* Linnaeus f. *molestus* Forskål (Diptera: Culicidae) from several areas of Russia

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**Abstract**

This is the first comparative study of the populations of the urban mosquito, *Culex pipiens* Linnaeus f. *molestus* Forskål, from the underground habitats from 10 cities of Russia (throughout the territory from 62-40°N, and 30-85°E). Some diagnostic morphometric, physiological and molecular characteristics were investigated. The mean siphonal index varied in geographical populations from 3.4 to 4.4 (i.e. within the reported range of the urban mosquitoes). Autogeny was recorded in all populations: the incidence of autogenous females varied from 60 to 100%. In all mosquito populations, 60-100% (mainly more than 90%) of females were infected with the endosymbiotic bacterium, *Wolbachia pipientis*. The *Wolbachia* infection was correlated with mitochondrial DNA haplotype (mitotype M). Two fragments, 220 bp and 90 bp in length, were obtained by means of restriction analysis of 3'end of cytochrome oxidase I (COI) gene using *Ssp I*. In all populations, the rDNA ITS2 fragments were c.460 bp (ITS2 = 325-333 bp). Similarity of the mitotypes and genotypes of the geographically isolated city populations support the hypothesis that the urban mosquitoes are of common origin.

**Keywords:** *Culex pipiens*, *Cx. pipiens* f. *molestus,* ITS2, autogeny

## Introduction

The urban mosquitoes *Cx. pipiens* Linnaeus f. *molestus* Forskål are of fundamental and applied importance as vectors, but their taxonomic status remains debated. At present, researchers are rather inclined to consider the urban mosquito to be an intraspecific form, a variety, eco-physiological race, ecotype or biotype of *Cx. pipiens* Linnaeus (Knight & Stone, 1978; Jupp, 1979; Harbach *et al*., 1985) or *Cx. p. pipiens* (Barr, 1981; Ishii, 1991; Vinogradova, 2000). Both *Cx. pipiens* and *Cx. pipiens* f. *molestus* differ little morphologically, but their biological features differ significantly. *Culex pipiens* f. *molestus* form is autogenous, stenogamous and anthropophilic, whereas the *pipiens* form is anautogenous, eurygamous and capable of diapause. Both forms occur in sympatry almost throughout the whole area of distribution (Vinogradova, 2000). In the temperate zone, both forms differ in their habitats. The urban mosquitoes develop throughout the year in underground sites flooded by polluted waters, e.g. in the basements of houses and underground tunnels. In this zone both forms are strongly isolated from one another. Such situation was observed in London, England (Byrne & Nichols, 1999) and in the Upper Rhein valley in Germany (Becker *et al.,* 1999). The degree of isolation between the two forms decreases in the southern aspects of the range, for instance, Egyptian populations of *Cx. pipiens* are relatively homogenous (Farid *et al.,* 1991).

*Culex molestus* Forskål was originally described from Egypt as a distinct species in 1775. It was recorded in Europe from the 1920s, first in London, England, and later in many other countries (Mihalyi, 1965; Vinogradova, 2000). Until 1992, underground larval habitats were recorded in more than 300 cities in the former USSR, but now they are much more widely distributed. This serious problem is connected with the unsatisfactory state of the housing and communal services in many Russian cities (Vinogradova, 2000). The increased density of mosquitoes associated with the urbanization process was predicted in 1964 at the WHO seminar on the ecology, biology and control of the *Culex pipiens* complex (WHO, 1965), and the developments since then confirm this trend.

Not only do urban mosquitoes cause nuisance, but they are effective vectors of human and animal diseases. Members of the Pipiens Complex are vectors of human lymphatic

filariasis and of many arboviral infections such as encephalitis (Western equine, St. Louis, Japanese, and West Nile), Rift Valley fever and Ockelbo disease (Vinogradova, 2000; Gratz, 2004). Attention is now focused on *Cx. pipiens* in connection with the recent spread of West Nile virus from Africa to North America and Europe. Large outbreaks of this disease occurred in 1996 in Romania and in 1999 in the USA (New York) and in Russia (Volgograd) (Petersen *et al*., 2002; Gratz, 2004). Additionally, in Slovakia the borreliae and hepatitis C viruses were isolated from these mosquitoes in Slovakia (Halouzka, 1993), and Egypt (Hassan *et al*., 2003), respectively.

*Culex pipiens* is a known host of the endosymbiotic bacterium *Wolbachia pipientis*. *Wolbachia* is a factor of various reproductive alterations in insects (Werren, 1997; Vinogradova *et al.,* 2002). The unidirectional cytoplasmic incompatibility between and within the members of the *Cx. pipiens* complex is connected with the fact that this bacterium is transmitted transovarially from the mother to its progeny (Yen & Barr, 1971). The distribution of *W. pipientis* in the natural populations of *Cx. pipiens* has not been adequately studied up until now.

The difficulty in the identification of various members of the *Cx. pipiens* complex within the limits of traditional taxonomy stimulated the development of their molecular genetic diagnostics (Crabtree *et al.,* 1995, 1997; Debrunner-Vossbrink *et al.,* 1996; Miller *et al.,* 1996; Severini *et al*., 1996; Kent *et al.,* 2003; Vinogradova *et al*., 2003; Smith & Fonseca, 2004; Vinogradova & Shaikevich, 2005; Shaikevich *et al*., 2005). Such studies are especially important in connection with different epidemiological roles of distinct members of the Pipiens Complex.

This paper is reports some morphometric, physiological and molecular characteristics of urban mosquito populations from the underground habitats in Russia.

## Materials and methods

Fourth instar mosquito were collected between 2001-2004 in the flooded basements of dwelling houses in 10 cities in Russia and also in Baku, Azerbaijan (Table 1). As a rule, the basements were flooded with sewage or mixed waters with a high organic content. Some larvae were preserved in 96% alcohol for further morphometric and molecular studies and some were used to obtain adults which were kept at 20°C and under short photoperiod (12 h of light per day) with sugar feeding; later the females were dissected to determine the expression of autogeny (the ability to produce eggs without blood meal).

The mean siphonal index of the larvae (the ratio of the siphon length to its width at the base) was used as a morphometric parameter for distinguishing between the populations of the *molestus* and *pipiens* forms (Vinogradova, 2000). Fifty larvae were studied in each case. Usual descriptive statistic (mean ± se) was used.

Mosquito DNA was extracted using DIAtom™ DNA Prep kit (Izogen, Moscow). Prior to DNA isolation from the alcohol-preserved material, alcohol was evaporated at 65° C for 30 to 40 min. Amplification was carried out using 0.1 μg of the isolated DNA. PCR was performed using GeneAmp R PCR System 2700 thermocycler (Applied Biosystems, United States) and the Universal (Izogen, Moscow) amplification kit.

Mitochondrial DNA was amplified using UEA9 and UEA10 primers (Juan *et al*., 1996), complementary to the 3'-end of the cytochrome oxidase I (COI) gene of *Drosophila yakuba*. For the identification of the endosymbiotic bacteria *Wolbachia pipientis* in mosquitoes, the *W. pipientis wsp*-specific primers of Braig *et al.* (1998) were utilized. The ribosomal ITS2 rDNA was amplified using the 5.8SF and 28SR primers listed in Porter & Collins (1991).

PCR conditions were identical for all pairs of primers and consisted of primary denaturation at 95°C for 5 min; then 35 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 45 sec; and a final extension at 72°C for 10 min. PCR products were identified using electrophoresis in 1% agarose gel (Sigma, United States). Amplified DNA fragments were isolated from the gel using DIAtom™ DNA elution reagent kit (Izogen, Moscow) and then directly sequenced. DNA sequencing was performed on an ABI PRISM 310 sequencer, using the Applera reagents kit (United States), according to the manufacturer's instructions. Sequences are available in GenBank under the following accession numbers: COI: AJ557889, AJ557891, AJ557892, AJ633083-AJ633085 & AY303550; ITS2: AJ850084-AJ850086.

For restriction analysis, the COI PCR product was digested with the enzyme *Ssp* I (Fermentas, Lithuania) for 1.5 h at 37°C. Reaction were carried out in a total volume of 50 μl and comprises 10μl COI PCR product, 1 μl *Ssp* I (10 U), 5 μl buffer G (10mM Tris-HCl (pH 7.4 at 25°C), 100mM KCl, 1mM DTT, 1mM EDTA, 0.2mg/ml BSA and 50% glycerol) and 34 μl

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| № | Locality | Coordinates N, E | Autogeny | | Infection by *Wolbachia* | | Mitotype | ITS2 (bp) | Siphonal index (mean± se) |
|---|---|---|---|---|---|---|---|---|---|
| | | | n= | % | N= | % | | | |
| 1 | Petrozavodsk I | 62°, 34° | ** | ** | 33 | 60 | M | 466 | 4.0±0.03 |
| 2 | Petrozavodsk II | 62°, 34° | ** | ** | 20 | 90 | M | 466 | 3.9±0.04 |
| 3 | St. Petersburg I | 60°, 30° | 54 | 89 | 50 | 98 | M | 463 | 4.2±0.03 |
| 4 | St. Petersburg II | 60°, 30° | 43 | 100 | 50 | 90 | M | 463 | 4.0±0.02 |
| 5 | Moscow | 56°, 38° | ** | ** | 53 | 96 | M | 462 | * |
| 6 | Nizhny Novgorod | 56°, 44° | 20 | 100 | 40 | 100 | M | 458,466 | 4.1±0.02 |
| 7 | Volgograd | 49°, 44° | 20 | 100 | 20 | 100 | M | 463 | * |
| 8 | Volzhsky | 49°, 45° | 17 | 100 | 17 | 88 | M | 463 | * |
| 9 | Krasnodar I | 45°, 39° | 30 | 90 | 20 | 70 | M | 463 | 4.4±0.03 |
| 10 | Krasnodar II | 45°< 39° | 55 | 49 | 20 | 98 | M | 463 | 3.4±0.02 |
| 11 | Baku | 40°, 50° | 50 | 89 | * | * | * | 463 | 4.0±0.03 |
| 12 | Ekaterinburg | 57°, 61° | * | * | 24 | 71 | M | 463 | * |
| 13 | Tomsk | 56°, 85° | * | * | 21 | 91 | M | 458,466 | * |

**Table 1**. Siphonal index of larvae, autogeny rate, infection by *Wolbachia pipientis*, mitotype and PCR products of ITS2 in the geographical populations of *Cx. pipiens* f. *molestus*. *data are absent, ** autogeny was recorded, but its quantity characteristic is absent.

dd$H_2O$. The results were visualized by the use of electrophoresis in 2% agarose gel.

## Results

Identification of the local populations was based on the ecological type of larval habitat), physiological (autogeny) and morphometric (mean siphonal index of larvae) characteristics. Most samples (12 of 13) were collected in underground water bodies, which are typical breeding places of the urban mosquitoes in the temperate zone. One local population (Krasnodar II) was collected in an over ground reservoir (an open system of sewage disposal). All populations appeared to be autogenous (Table 1). The incidence of autogenous females in most cases was high (89-100%) and decreasing to only 49% among mosquitoes from Krasnodar II. The mean siphonal index of local populations varied within 3.4-4.4. Within each sample, the distribution of the individual siphonal index was normal or about normal.
*Wolbachia pipientis* infections were noted in all populations, where the proportion of infected individuals varied from 60 to 100% However infection rates were >90% in most populations (Table 1).

Comparative analysis of 247 nucleotides of the 311bp fragment of the 3'end of the COI gene shows the 100% identity in all populations. Twenty individuals were surveyed from nine urban populations, excluding Baku, using restrictase *Ssp* I. Results confirmed the mitotype as that named earlier as type M (Shaikevich *et al.,* 2005) was common in all studied populations. Table 1 show that this mitochondrial haplotype is positively correlated with *W. pipientis* infection. In mosquitoes from St. Petersburg, the COI sequences were identical both in individuals infected and uninfected with *Wolbachia*.

Comparison of the nuclear ITS2 sequences revealed length polymorphism, with PCR products varying between 458-466 bp in length (ITS2 = 325-333bp). ITS2 of two specimens from the Tomsk and N. Novgorod populations (Tomsk-1 and Tomsk-2, N. Novgorod-1 and N. Novgorod-2) were sequenced to determine intrapopulation variation. The fragments of ITS2 sequences differ in length in local populations: 325 bp from N. Novgorod-1 and Tomsk-2, 329 bp from Moscow, 330 bp from St. Petersburg I, 332 bp from Petrozavodsk II and 333 bp from N. Novgorod-2 and Tomsk-1 (Figure 1). Such differences were observed both within the same population (N. Novgorod-1 and -2) and between populations, for instance, N. Novgorod and St Petersburg I. Pairwise alignment of ITS2 sequences showed 1-4% sequence differences. Maximum similarity (99%) was observed in the ITS2 sequences of mosquitoes in St. Petersburg I and Moscow, Moscow and Tomsk-2, Tomsk-2 and N. Novgorod-1, Petrozavodsk II and Tomsk-1. Minimum similarity (96%) was recorded in the ITS2 sequences in mosquitoes from Moscow and Tomsk-1, Petrozavodsk II and Moscow. Such variation of the ITS2 sequences in *Cx. pipiens* f. *molestus* from Russia corresponds to that in *Cx. pipiens* from the USA (Miller *et al.,* 1996). The ITS2 sequences of the mosquitoes from St. Petersburg I correspond (98%) to those

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Petersburg I  181 GGGGTTTTCGTTCGGCGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
Moscow            GGGGTTTTCGTTCG-CGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
Tomsk-2           GGGGTTTTCGTTCG-CGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
N.Novgorod-1      GGGGTTTTCGTTCG-CGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
N.Novgorod-2      GGGGTTTTCGTTCGGCGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
Tomsk-1           GGGGTTTTCGTTCGGCGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
Petrozavodsk II   GGGGTTTTCGTTCG-CGGACGGCCACACTGGTGCGCACGCACGCGACTGAACGGACGACG
                  ************** ******************************************

Petersburg I  241 ACG---GTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
Moscow            ACG---GTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
Tomsk-2           ACG---GTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
N.Novgorod-1      ACG---GTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
N.Novgorod-2      ACGACGGTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
Tomsk1            ACGACGGTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
Petrozavodsk II   ACGACGGTGAGAATACATCCCACACACCAACCTGGCTTGGGCGCCGATGTAGCATCTCTC
                  ***   ****************************************************

Petersburg I  301 ACGCCACGT--CGTCGTCGTCACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
Moscow            ACGCCACGT--CGTCGTCGTCACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
Tomsk-2           ACGCCACGT--CGTCGT---CACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
N.Novgorod-1      ACGCCACGT--CGTCGT---CACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
N.Novgorod-2      ACGC--CGTCACGTCGTCGTCACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
Tomsk-1           ACGC--CGTCACGTCGTCGTCACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
Petrozavodsk II   ACGC--CGTCACGTCGTCGTCACACGTTCGTTCGGTCATCCGGCGTCGTCGCGGTACCGC
                  ****  ***  ******   ****************************************

Petersburg I  361 GTCCACAGAACAGAACAACCCAACACACGAGCA-GCG-ATCGACAAGCGATAAGATAAAC
Moscow            GTCCACAGAACAGAACAACCCAACACACGAGCATGCG-ATCGACAAGCGATAAGATAAAC
Tomsk-2           GTCCACAGAACAGAACAACCCAACACACGAGCA-GCG-AACGACAAGCGATAAGATAAAC
N.Novgorod-1      GTCCACAGAACAGAACAACCCAACACACGAGCA-GCG-AACGACAAGCGATAAGATAAAC
N.Novgorod-2      GTCCACAGAACAGAACAACCCAACACACGAGCA-GCG-ATCGACAAGCGATAAGATAAAC
Tomsk-1           GTCCACAGAACAGAACAACCCAACACACGAGCA-GCGCATCGACAAGCGATAAGATAAAC
Petrozavodsk II   GTCCACAGAACAGAACAACCCAACACACGAGCA-GCGCATCGACAAGCGATAAGATAAAC
                  ********************************* *** * ********************

                ITS2←
Petersburg I  421 CCCCCATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
Moscow            CCCCCATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
Tomsk-2           CCCC-ATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
N.Novgorod-1      CCCC-ATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
N.Novgorod-2      CCCCCATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
Tomsk-1           CCCCCATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
Petrozavodsk II   CCCCCATGTAGGCCTCAAATAATGTGTGACTACCCCCTAAATTTAAGCAT
                  **** ******************************************
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**Figure 1:** Alignment of *Cx. p. pipiens* f. *molestus* ITS2 nucleotide sequences (partial, from 181bp to the end) including 28S rDNA region and underlined primer annealing site. DNA of the first half of ITS2 sequences are identical. Sequences are available in GenBank under accession numbers AJ850084-AJ850086.

of the *Cx. p. molestus* clone PMA6J32 from the USA (CPU22116).

## Discussion

We carried out the first integrated study of geographic populations of the urban mosquitoes originating from underground habitats in ten cities over the territory of Russia. The main diagnostic morphometric, physiological and molecular characteristics of the local populations were investigated. The expression of autogeny was confirmed in 11 populations, and high expression of autogeny (90-100%) was revealed in most cases. In St. Petersburg, this phenomenon was recorded as long ago as 1987 (Vinogradova & Oda, 1990) and later in 1997-2001 when in 10 of 14 populations the fraction of autogenous females was about 100% (Vinogradova, 2001). It is known that autogeny, being a genetically determined character, depends on the conditions of larval development, in particular on larval feeding. A high level of organic pollution promoting the expression of autogeny was observed in all underground habitats of mosquitoes in Russian cities. Only one population of the autogenous form (Krasnodar-II) inhabited an over ground polluted reservoir connected with an underground habitat

where mosquitoes reproduced the whole year round. Homogenous populations of *Cx. pipiens* f. *molestus* form or mixed populations (with the *Cx. pipiens*) were earlier reported from over ground reservoirs, mainly in the southern regions of the former USSR, Hungary, Poland, USA and Japan (Vinogradova, 2000).

In all local populations the mean siphonal index of larvae corresponded to that of the typical *molestus* form, being 4.4 or less, whereas in the *pipiens* form it is usually 4.8 and more (Vinogradova, 2000). A significant variation of the mean siphonal indices was established both within and between geographic populations of mosquitoes. In 1987, similar values were recorded in several subpopulations from St. Petersburg being 3.9, 4.2, 4.4 and 4.4 (Vinogradova & Oda, 1990). In general, significant variation of the siphonal index is typical of both *Cx. pipiens* and *Cx. pipiens* f. *molestus* (Vinogradova, 2000).

Almost all larvae were tested for *Wolbachia pipientis* infection. This cytoplasmic symbiotic bacterium was found in all studied populations, at average levels of 90% or more. There are not many literature records on the distribution of *W. pipientis* among various members of the *Culex pipiens* complex. It was previously found in *Cx. pipiens* from England, in *Cx. pallens* Coquillett from Japan, in members of the complex of the *Culex pipiens* complex from Australia (Irving-Bell, 1983; O'Neil *et al.,* 1992), in hibernating *Cx. pipiens* and *Cx. torrentium* from Sweden (Larsson, 1983), in three strains of *Cx. pipiens* from USA, California, and in *Cx. quinquefasciatus* from Skukuza in South Africa, where it was absent in *Cx. pipiens* (Cornel *et al*., 2003).

The sequences of *wsp* genes of *W. pipientis* in the urban mosquitoes from St. Petersburg, Moscow and Volzhsky (Vinogradova *et al*., 2003) were identical with those which were revealed in *Cx. pipiens* from Italy (AJ311043), Tunis (AF020061), California, USA (AF301010), China (AF216860), *Cx. p. pallens* (AF216860), *Cx. quinquefasciatus* (AY462861), *Cx. torrentium* (AJ311041), *Cx. modestus* Filcalbi (AJ311042) and *Aedes punctor* (Kirby) (AJ311040). The *wsp* gene of *Wolbachia* is considered highly variable and can be used to resolve the evolutionary relationships between different *Wolbachia* strains (Zhou *et al.,* 1998). However this fragment is not informative for the analysis of the differences between bacterial strains infecting mosquitoes of the genus *Culex* or understanding the mechanisms of cytoplasmic incompatibility induced by this bacterium.

The COI sequences are useful diagnostic markers for the differentiating many insect species (Juan *et al.,* 1996; Bernasconi *et al*., 2000). The COI sequence of the *Cx. pipiens* f. *molestus* differs from *Cx. torrentium* (AJ633091) by 2.5%, *Culex tarsalis* Coquillett (AF425847) by 4%, *Culex descens* Theobald (AY645241) by 8%, and from *Culiseta impatiens* (Walker) (AF425848) and *Aedes aegypti* Linnaeus (AY645271) by 11%. Thus, the COI gene may be a useful marker for taxonomic and phylogenetic studies of mosquitoes, but the 3' end of the gene showed little variation within *Cx. pipiens* f. *molestus* populations from nine cities in Russia.

The ITS2 region is known to be highly variable in contrast to the conserved regions encoding the 5.8S and 28S rDNA genes. According to our results, DNA of the first half of ITS2 is less variable than the second half. Comparing the ITS2 sequences in the region of nucleotide 243 we revealed differences in the number of ACG repeats (Figure 1). An indel of three bases in a microsatellite repeat is observed (insertion of ACG at bases 244-246) in specimens from N. Novgorod-2, Tomsk-1 and Petrozavodsk II. Additionally, variation in the number of CGT repeats alternating with CA repeats is noted in the region between nucleotides 305 and 330. In all studied sequences, CGT is also repeated twice in the region of nucleotide 344 and one time in the region of nucleotide 360. This may signify to a high recombination activity of this region of ITS2. Single nucleotide indels are also observed in other cases.

A high similarity in the nucleotide composition of ITS2 (99%) was revealed in the mosquito populations remote from each other: Moscow and Tomsk, Petrozavodsk II and Tomsk, Tomsk and N. Novgorod, St. Petersburg I and Moscow; two specimens of the same population (N. Novgorod and Tomsk) differ (3%) in the composition of ITS2. The mutation processes in the region of the variable spacer of rDNA in *Cx. pipiens* probably occur in different populations independently of their habitats.

Molecular differences between autogenous and anautogenous *Cx. pipiens* are of interest for taxonomy and the estimation of microevolutionary processes. The comparison of the underground populations (*Cx. pipiens* f. *molestus*) from Moscow and over ground populations (Cx. *pipiens*) from around Moscow showed their 100% identity in their ITS2 and the 3' end of the COI gene (Vinogradova & Shaikevich, 2005; Fyodorova & Shaikevich, 2007). However, differences have been exposed between autogenous and anautogenous forms

using other region of COI gene (Shaikevich, 2007).

These molecular data may be of interest in connection with the origin of the autogenous form in temperate zones, in particular in Europe. Two hypotheses are discussed in the literature (Kitzmiller, 1976; Barr, 1981; Byrne & Nichols, 1999; Vinogradova, 2000; Smith & Fonseca, 2004). One hypothesis suggests that northern European underground populations are derived from Mediterranean autogenous populations of southern Europe and northern Africa, which comprise a monophyletic group. Alternatively, northern autogenous populations could be derived from the anautogenous over ground local populations as a result of adaptation to underground environments. Our results showing the similarity of the mitotypes and genotypes of all autogenous populations from underground habitats in ten geographically distinct Russian cities may support the common origin of the urban mosquitoes.

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