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**ANIMAL GENETICS**

# Chromosomal Polymorphism in the Populations of Malaria Mosquito *Anopheles messeae* (Diptera, Culicidae) in the Volga Region

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**Abstract**—We studied the species composition and chromosomal variability of malaria mosquitoes in the Volga Basin (Upper, Middle, and Lower Volga regions). We investigated larvae karyotypes of sibling species of the *Anopheles maculipennis* group. We calculated the frequencies of chromosomal inversions in the local populations of the dominant species *An. messeae*. We discovered that karyotypic structure of *An. messeae* populations depends on landscape-climatic zones. Populations of the Upper, Middle and Lower Volga differ in frequency of chromosome inversions XL, 2R, 3R, and 3L.

*Keywords*: chromosomal polymorphism, malaria mosquitoes, landscape-climatic zones, *Anopheles*
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## INTRODUCTION

The study of genetic mechanisms of formation of population-genetic system of a species is a major direction of research in contemporary population genetics. In conditions of environmental crisis and degradation of ecosystems, analysis of population-genetic systems of thriving species that can exist in natural and anthropogenically modified landscapes is of particular interest. An example of such thriving species is malaria mosquitoes of the *Anopheles maculipennis* group. In the CIS countries, this group consists of ten sibling species, which mastered most of the Palaearctic area up to the northern border of the taiga zone. After cessation of antimalarial activities in the 1950s, populations of malaria mosquitoes regained their strength and adapted to life in an urban environment. One of the most common species living alongside humans is malaria mosquito *An. messeae* Fall., 1926. Populations of *An. messeae* show a high level of chromosomal variability, which probably allowed mosquitoes of these species to occupy the most extensive range in the Palearctic.

In the European part of Russia, a large part of *An. messeae* habitat is located in the basin of the Volga River. The Volga area includes different landscape zones with different types of habitats of malaria mosquitoes [1]. This region is poorly investigated in respect to geographical distribution and genetic structure of mosquito populations of the *Anopheles maculipennis* complex. The goal of this study was to investigate the chromosomal composition of *An. messeae* populations within different landscape-climatic zones in the territory of the Volga region.

## MATERIALS AND METHODS

Samples of fourth stage larvae of malaria mosquitoes were collected in 2013–2014 in eight localities within the basin of Volga region. Location of larval habitats, collection dates, and number of individuals in sample groups are listed in Table 1. Samples from different habitats were divided into three groups according to the landscape zoning of the region [1]:

(1) Upper Volga region (Upper Volga): region of southern taiga and mixed coniferous-deciduous forests (taiga and subtaiga zone);

(2) Middle Volga region: zone of deciduous forests and steppe;

(3) Lower Volga region: steppe and semidesert areas.

It should be noted that boundaries of these landscape areas are rather conventional and include transitional areas. In the region of Nizhny Novgorod conifers, pine forests with bogs are replaced by oak and spruce forests and pine forests. The Samara region contains the boundary between the southern forest steppe and steppe. South of Volgograd, steppe transitions to semidesert, and in Astrakhan oblast, it transitions to a desert.

Fourth stage larvae of *Anopheles mosquitoes* caught in different habitats were fixed in alcohol–vinegar mixture (3 : 1). The diagnostics of malaria mosquitoes was carried out on the basis of morphological [2, 3] and cytogenetic characteristics. Salivary glands were used to prepare colored samples of polytene chromosomes using a standard lacto-aceto-orcein methodology [4]. Karyotypes of polymorphic *An. messeae* spe-

**Table 1.** Species composition of investigated malaria mosquitoes in Volga habitats

| No. | Habitat, GPS coordinates | Date of capture | Number of individuals | Dominance index between sibling species, $f \pm s_f$, % | | |
|---|---|---|---|---|---|---|
| | | | | *An. messeae* | *An. maculipennis* | *An. beklemishevi* |
| Upper Volga | | | | | | |
| 1 | Tver oblast, Rzhev district, Gorki village 56°27′15.29″ N 33°53′18.24″ E | 05.08.2013 | 122 | 97.6 ± 1.4 | 1.6 ± 1.1 | 0.8 ± 0.8 |
| Middle Volga | | | | | | |
| 2 | Nizhny Novgorod, Fedoseenko Street area 56°20′0.84″ N 43°48′13.53″ E | 16.07.2013 | 121 | 95.9 ± 1.8 | 4.1 ± 1.8 | 0 |
| 3 | Chuvash Republic, Cheboksary 56°7′42.16″ N 47°20′30.51″ E | 17.07.2013 | 94 | 94.7 ± 2.3 | 5.3 ± 2.3 | 0 |
| 4 | Republic of Tatarstan, vicinity of Kazan 55°53′13.17″ N 48°52′3.96″ E | 17.07.2013 | 105 | 100 | 0 | 0 |
| 5 | Republic of Tatarstan, Naberezhnye Chelny 55°41′4.84″ N 52°29′23.06″ E | 18.07.2013 | 111 | 99.1 ± 0.9 | 0.9 ± 0.9 | 0 |
| 6 | Republic of Bashkortostan, Ilishevsky district, Zyaylevo village 55°28′29.94″ N 54°2′0.89″ E | 18.07.2013 | 132 | 77.3 ± 3.6 | 22.7 ± 3.6 | 0 |
| Lower Volga | | | | | | |
| 7 | Samara, Lake Yaickoe 53°6′13.97″ N 50°12′52.95″ E | 22.07.2014 | 105 | 100 | 0 | 0 |
| 8 | Volgograd oblast, Novogrigorevskaya village, Lake Melkoe 49°26′55.96″ N 43°36′37.77″ E | 26.07.2014 | 83 | 100 | 0 | 0 |

cies were analyzed for homo- and heterozygosity on the basis of paracentric inversions [5]. *An. messeae* populations were examined for frequencies of inversions. In total, 873 larval karyotypes were investigated.

## RESULTS AND DISCUSSION

Results of cytogenetic analysis showed that the Volga region is inhabited by three sibling species of the *Anopheles maculipennis* complex: *An. beklemishevi* Stegn. et Kabanova, 1976; *An. maculipennis* Meig., 1818; and *An. messeae* Fall., 1926 (Table 1). The northern border of the habitat of malaria mosquito *An. beklemishevi* lies in the Upper Volga region. In fact, the southernmost habitat of this species was found in the Rzhev district in Tver oblast. Earlier reports indicated occurrence of *An. beklemishevi* individuals in more southern regions, in particular, in the north of Moscow oblast [6]. According to current data, this species does not appear in Moscow oblast

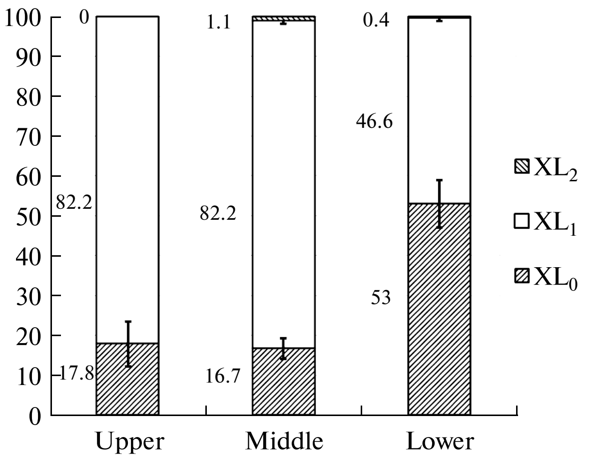


**Fig. 1.** Frequency of inversions of sex chromosome XL in populations of mosquito *An. messeae* in the Upper, Middle, and Lower Volga. Hereinafter, the *y* axis represents the frequency of inversions (in %). Shown are 95% confidence intervals.

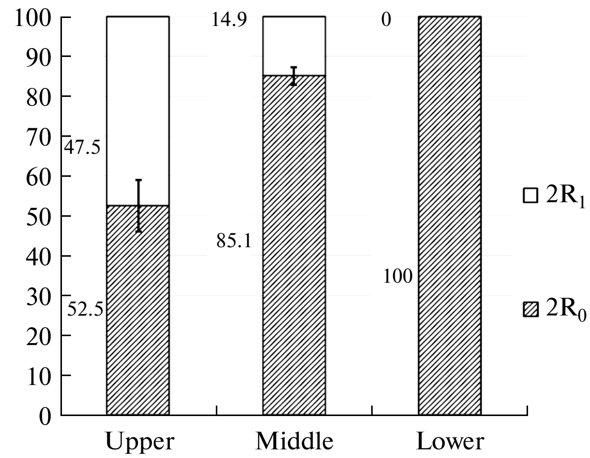


**Fig. 2.** Frequency of inversions of chromosome 2R in populations of mosquito *An. messeae* in the Upper, Middle, and Lower Volga.

[7]. Habitats of mosquito *An. maculipennis* were found on the territory of the Upper and Middle Volga region. As a rule, larvae development of this species occurs along with *An. messeae* individuals. The latter species dominates everywhere, and isolated breeding sites of *An. maculipennis* can be found only in temporary habitats [7]. The Lower Volga region is dominated by *An. messeae*. It should be noted that, in Volgograd oblast, only places of mass breeding of malaria mosquitoes in freshwater bodies were examined. In such habitats, the probability of detection *An. maculipennis* was low. Besides the two mentioned species, brackish lakes of desert and semidesert areas of the Lower Volga are home to another representative of the *maculipennis* complex—malaria mosquito *An. atroparvus* Van Thiel, 1923 [3]. Mosquitoes of this species have been detected in Astrakhan oblast and Kalmykia [8, 9]. The eastern boundary of the range of malaria mosquito *An. atroparvus* has not been determined.

Mosquito *An. messeae*, unlike other species, manifests a high level of inversion polymorphism in populations of the Volga region (Table 2). Data on inversion frequencies for XL sex chromosome homo- and heterozygotes are shown separately for males and females; data on composition of autosomes 2R, 3R, and 3L are shown together for both sexes. The frequencies of inversions change greatly when moving from the upper to the lower reaches of the Volga, from one landscape to another. Populations of the Upper and Middle Volga region located in the taiga zone did not differ in the frequencies of sex chromosome inversions, with one exception: a rare inversion in $XL_2$, which appears in Kazan and other habitats in down the Volga (Table 2). Individuals with this inversion occur with increased frequency in populations of Western Siberia. Until now, this inversion was considered endemic to Siberia. In comparison with the Middle Volga region, populations of the Lower Volga basin show a sharp almost twofold increase in the frequency of $XL_0$ inversion, which predominates in the southwest of the species range (Fig. 1; $\chi^2 = 143.7$; the number of degrees of freedom *d.f.* = 2; $p < 0.001$). In our opinion, the increase in the frequency of this inversion in the Lower Volga region is associated with transition from the forest-steppe to steppe landscape-climatic zone.

Populations of *An. messeae* show significant change in the composition of autosomes 2R, 3R, and 3L (Figs. 2–4) when moving from the upper to lower riverbed. The frequency of $2R_1$ inversion consistently declined, and in the Lower Volga region, the share of alternative sequence $2R_0$ reached 100% (Fig. 2; $\chi^2 = 182.7$; *d.f.* = 2; $p < 0.001$). Thus, our observations confirm a clinal nature of variability for $2R_1$ inversion in the Volga basin, noted in previous studies [5, 10]. The proportion of mosquitoes with inversion $3R_1$ did not change in the upper and middle reaches of the Volga, but significantly decreased in the Lower Volga region (Fig. 3, $\chi^2 = 28.1$; *d.f.* = 2; $p < 0.001$) with transition to the steppe zone. Frequency of "southern" inversion $3L_1$ is minimal in the taiga zone (in the Upper Volga region), but significantly increased in the forest-steppe and steppe zones (in the Middle and Lower Volga regions) (Fig. 4; $\chi^2 = 11.1$; *d.f.* = 2; $p < 0.001$). Thus, transition from one landscape-climatic zone to another is manifested in restructuring of the karyotypic structure of populations, which affects inversion of all chromosomes.

It is worth discussing the problem of how landscape zones are able to influence chromosomal composition of *An. messeae* populations. Undoubtedly, conditions of vital activity of mosquitoes depend on the basic landscape factors, such as temperature and rainfall. Yet, apart from direct influences of climatic factors on the insect organism, there are also indirect effects. For

**Table 2.** Chromosome structure of local populations of mosquito *An. messeae* in different regions of the Volga region

| Chromosomal variants | Frequencies of chromosomal variants, $f \pm s_f$, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Upper Volga | Middle Volga | | | | | Lower Volga | |
| | Gorki village, Rzhevsky district, Tver oblast | Nizhny Novgorod | Cheboksary | Kazan | Naberezhnye Chelny | Zyaylevo village, Bashkortostan | Samara | Volgograd oblast, Novogrigor-evskaya village |
| Males, *n* | 58 | 66 | 35 | 41 | 52 | 56 | 50 | 43 |
| $XL_0$ | 13.8 ± 4.5 | 36.4 ± 5.9 | 20.0 ± 6.8 | 0 | 5.8 ± 3.2 | 1.8 ± 1.8 | 70 ± 6.5 | 39.5 ± 7.5 |
| $XL_1$ | 86.2 ± 4.5 | 63.6 ± 5.9 | 80.0 ± 6.8 | 100 | 94.2 ± 3.2 | 94.6 ± 3.0 | 28 ± 6.3 | 60.5 ± 7.5 |
| $XL_2$ | 0 | 0 | 0 | 0 | 0 | 3.6 ± 2.5 | 2.0 ± 2.0 | 0 |
| Females, *n* | 61 | 50 | 54 | 64 | 58 | 46 | 55 | 40 |
| $XL_{00}$ | 11.5 ± 4.1 | 22.0 ± 5.0 | 14.8 ± 4.8 | 1.5 ± 1.6 | 3.5 ± 2.4 | 4.4 ± 3.0 | 27.3 ± 6.0 | 25.0 ± 6.8 |
| $XL_{01}$ | 16.4 ± 4.7 | 16.0 ± 4.5 | 16.7 ± 5.1 | 14.1 ± 4.3 | 6.9 ± 3.3 | 6.5 ± 3.6 | 49.1 ± 6.7 | 52.5 ± 7.9 |
| $XL_{11}$ | 72.1 ± 5.7 | 60.0 ± 6.0 | 68.5 ± 6.3 | 79.7 ± 5.0 | 87.9 ± 4.3 | 82.6 ± 5.6 | 23.6 ± 5.7 | 22.5 ± 6.6 |
| $XL_{12}$ | 0 | 0 | 0 | 4.7 ± 2.6 | 1.7 ± 1.7 | 6.5 ± 3.6 | 0 | 0 |
| $XL_{14}$ | 0 | 2.0 ± 4.9 | 0 | 0 | 0 | 0 | 0 | 0 |
| Both sexes, *n* | 119 | 116 | 89 | 105 | 110 | 102 | 105 | 83 |
| $2R_{00}$ | 30.0 ± 1.9 | 76.7 ± 3.9 | 67.4 ± 5.0 | 69.5 ± 4.5 | 70.9 ± 4.3 | 82.3 ± 3.8 | 100 | 100 |
| $2R_{01}$ | 45.5 ± 2.3 | 18.1 ± 3.5 | 30.3 ± 4.9 | 24.8 ± 4.2 | 26.4 ± 4.2 | 16.7 ± 3.7 | 0 | 0 |
| $2R_{11}$ | 24.5 ± 1.7 | 5.2 ± 4.6 | 2.3 ± 1.6 | 5.7 ± 2.3 | 2.7 ± 1.6 | 1.0 ± 1.0 | 0 | 0 |
| $3R_{00}$ | 62.5 ± 2.2 | 70.7 ± 4.2 | 55.1 ± 5.3 | 53.3 ± 4.9 | 47.3 ± 4.8 | 33.3 ± 4.7 | 72.4 ± 4.4 | 74.7 ± 4.8 |
| $3R_{01}$ | 30.5 ± 2.0 | 24.1 ± 3.9 | 40.4 ± 5.2 | 40.0 ± 4.8 | 42.7 ± 4.7 | 52.0 ± 4.9 | 25.7 ± 4.3 | 24.1 ± 4.7 |
| $3R_{11}$ | 7.0 ± 0.6 | 5.2 ± 4.6 | 4.5 ± 2.2 | 6.7 ± 2.4 | 10.0 ± 2.9 | 14.7 ± 3.5 | 1.9 ± 1.3 | 1.2 ± 1.2 |
| $3L_{00}$ | 95.0 ± 0.4 | 94.0 ± 2.2 | 93.3 ± 2.7 | 81.9 ± 3.8 | 75.5 ± 4.1 | 79.4 ± 4.0 | 84.8 ± 3.5 | 80.7 ± 4.3 |
| $3L_{01}$ | 4.2 ± 1.8 | 6.0 ± 4.5 | 6.7 ± 2.7 | 18.1 ± 3.8 | 22.7 ± 4.0 | 19.6 ± 3.9 | 15.2 ± 3.5 | 19.3 ± 4.3 |
| $3L_{11}$ | 0.8 ± 0.8 | 0 | 0 | 0 | 1.8 ± 1.3 | 1.0 ± 1.0 | 0 | 0 |

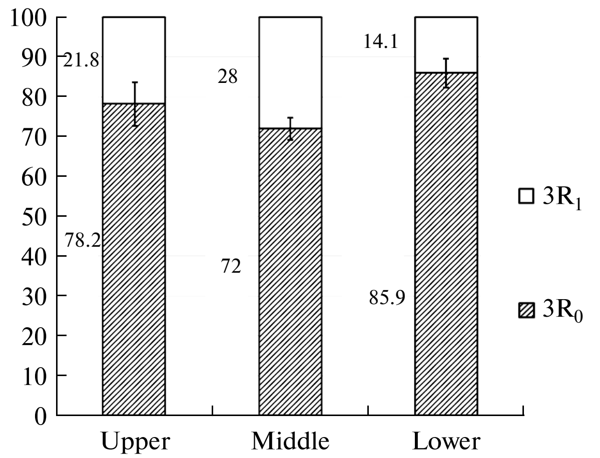


**Fig. 3.** Frequency of inversions of chromosome 3R in populations of mosquito *An. messeae* in the Upper, Middle, and Lower Volga.

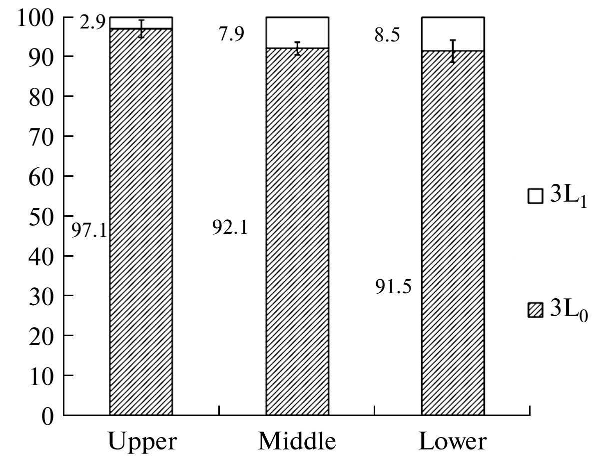


**Fig. 4.** Frequency of inversions of chromosome 3L in populations of mosquito *An. messeae* in the Upper, Middle, and Lower Volga.

example, vegetation is an important component of landscapes. In a forest zone, the living conditions of the imago are completely different than in the steppe or desert. Woody vegetation prevents mosquitoes from overheating in summer and allows diapause females to survive the winter. It is possible that, in the steppe zone, mosquitoes with "northern" inversion $2R_1$ are not able to reach a sufficient number of diapausing imagoes before the onset of winter to provide for the massive development of larvae in the spring. In summer, during periods of population "prosperity," individuals with $2R_0$ inversion colonize all favorable habitats and displace mosquitoes with $2R_1$ inversion. In the taiga zone, there are conditions for development of mosquitoes with different chromosomal rearrangements. However, in the north of that zone, individuals with $2R_0$ inversion are not able to tolerate freezing winter temperatures. Thus, it is not surprising that, in the context of climate warming, *An. messeae* in the north of the area manifests an increase in the frequency of "southern" inversions. For example, currently, mosquitoes with the $2R_0$ and $XL_0$ chromosomes are detected in the basin of the Vychegda River (Republic of Komi) and in the north of Tomsk oblast [11, 12]. Earlier, in the 1970s, there were no individuals with "southern" inversions in these populations [5].

Thus, we believe that climate change can affect the geographic distribution and chromosomal composition of malaria mosquitoes in the Volga region. Global warming can lead to a change in the borders of landscape-climatic zones and a shift in the northern borders of the sibling species of malaria mosquitoes. Notably, the first sign of the impending change in the communities will be an alteration in karyotypic structure of populations of polymorphic species such as *An. messeae*. Undoubtedly, change in the succession series is a slower process than rearrangement of the population genetic structure. Thus, conducting population-genetic monitoring makes it possible to detect the initial stages of directed transformation of ecosystems.

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