Comparative analysis of the variability of the genetic structure of *Anopheles messeae* (Diptera, Culicidae) populations from Western Siberia and Eastern Kazakhstan

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**Abstract.** A comparison of the species composition of anopheles mosquitoes from the East Kazakhstan region and the south of Western Siberia has been carried out. A comparative cytogenetic analysis of *An. messeae* larvae from the southern and central parts of the range was done. The tendency of displacement of the “south-eastern” genocomplexes from the center of the *An. messeae* habitat to its southern borders has been observed.

**1. Introduction**

Chromosomal variability is common in various representatives of Diptera. Inversions are the most common type of chromosomal rearrangements in Diptera, when a segment of chromosomes is inverted at 180 degrees. Inversion polymorphism was found in a significant number of insect species in such families as Culicidae, Chironomidae, Simuliidae. It is confirmed that inversions play an important adaptive role [1, 2]. Among these families, malaria mosquitoes of *Anopheles* genus, family Culicidae, are of considerable interest in inversion polymorphism. The peculiarity of malaria mosquitoes is the presence of ecologically specialized twin species that differ in the level of chromosomal variability. Worldwide interest in the genus *Anopheles* stems from its inauspicious role in the transmission of malaria, responsible for over 1 million deaths per year [3].

The Anophles mosquito *Anopheles messeae*, among other cryptic species of the *Anopheles maculipennis* complex, has the largest habitat, covering almost the entire boreal subregion of the Palearctic. In the populations of *An. messeae*, five widespread fluctuating chromosomal inversions existing in hetero- and homozygous states were revealed [4]. When studying chromosomal polymorphism in the anophesles mosquito *An. messeae*, it was found that there is a clinal distribution of inversion frequencies from west to east and from south to north [4, 5]. The frequency of inversions in the *An. messeae* populations correlates well with the climatic and environmental conditions of mosquitoes' habitat and reflects the clinal character.

XL\(_1\) and 3R\(_1\) inversions have a clear (for 3L\(_1\) less pronounced) clinal variation of frequencies in the longitudinal direction, and 2R\(_1\) inversions – in the latitudinal direction [6]. It was established that chromosomal polymorphism is supported by the differential adaptability of alternative homozygotes at different stages of the seasonal cycle. For individuals, identified in populations with “northern” and
“southern” cytotypes, the difference in a number of adaptive traits was noted: fecundity and viability of adults, larvae competitiveness, sensitivity to parasites and pathogens, resistance of larvae to asphyxiation (acute oxygen starvation) and lack of food, the effects of temperature and other abiotic factors [4, 7].

In climate warming conditions, An. messeae populations from the northern part of Western Siberia and northern Europe show a high level of "southern" inversions, previously not registered in these populations [4, 5, 8]. In populations of Western Siberia, such processes began to occur in 1983 [4, 9]. After 2014, the same changes were noted for the An. messeae species in populations from the southern boundary of the habitat in the Republic of Kazakhstan [10]. At the same time, the northern boundary of the mosquito An. maculipennis habitat shifted to higher latitudes with simultaneous distribution of these species to the east, from the Volga region to the southern Urals, to the Chelyabinsk and Orenburg regions [8].

The described processes occurring in populations of mosquito An. maculipennis within their natural habitats are of great interest in studying genetics and evolution. Timely delineation of An. maculipennis habitat boundaries is important for ecological monitoring of malaria mosquito populations.

Capturing genetic structure changes in An. messeae populations that happen due to adaptation of mosquitoes to changing environmental conditions requires an updating information on the species composition of anopheles mosquitoes and chromosomal polymorphism study in these populations in latitudinal direction from Eastern Kazakhstan (southern boundary of the habitat) to Western Siberia (center of the habitat). This is also important in connection with their epidemiological significance, as carriers of malaria. Features of climatic preferences, behavior, physiology of various types of mosquitoes and polymorphic forms within the same species require the use of different control strategies [11, 12].

2. Material and methods
The object of the study was the fourth stage larvae of malaria mosquitoes. The location of larval biotopes, the dates of collection and the number of individuals in the samples are shown in Table 1.

The captured larvae were fixed with an alcohol-acetic mixture (3:1) for further morphological and cytogenetic analysis. Diagnostics of mosquitoes was performed according to morphological features [13].

Temporary preparations of polytene chromosomes were done in laboratory conditions using the standard lactoacetorsein method [14]. Homo- and heterozygotes for paracentric inversions in the karyotypes of the genetically polymorphic species An. messeae were determined using the appropriate chromosome maps [4]. A total of 144 individuals were cytogenetically studied.

3. Results and Discussion
During the morphological analysis of the species composition in populations from Eastern Kazakhstan, we detected anopheles mosquitoes An. messeae, An. claviger and An. hyrcanus (Zharbulak village – (10.08.2017) 46° 09‘29“ N 82° 03‘09“ E). In the population from Kolarovo village (Tomsk region) in the south of Western Siberia, two species of An. messeae Fall. and An. beklemishevii were detected (Table 1). Based on the morphological analysis, we previously found that in the populations from the Kuznetsk Alatau, the ratio of mosquitoes An. beklemishevii and An. messeae differs from other regions of Siberia [15]. In these populations, mosquitoes of the species An. beklemishevii are represented in the majority and their number varies from 97% to 100%. In Eastern Kazakhstan, An. beklemishevii specie was not found; however, based on the mass presence in the Kuznetsk Alatau, this species can also live on the border of Gorny Altai. In different regions, the ratio of the number of larvae of these species in water bodies varies during the season, and also depends on the physical properties, chemical composition as well as saprobity of water [16, 17].

Larvae of the An.claviger and An.messeae species were found in springs in two populations from Eastern Kazakhstan (Table 1).
In addition to studying the species composition of malaria mosquitoes, we also conducted a study of the chromosome composition of the *An. messeae* populations in six populations from Eastern Kazakhstan. Data on the ratio of the inversion homo- and heterozygotes of the sex chromosome XL are given separately for males and females; data on the composition of autosomes 2R, 3R and 3L – for individuals of both sexes (Table 2). The obtained data were compared with the literature data for *An. messeae* in Western Siberia, to confirm the adaptive nature of inversions caused by changing climatic conditions.

Karyotypes, which combine inversion variants of one of the associative groups, were called “genocomplexes” [4, 18, 19]. Different genocomplexes substitute each other at the periphery of the habitat, but in most populations in the center of habitat they occur together.

**Table 1.** Species composition of investigated malaria mosquitoes.

| No. | Habitat                                      | Date of capture, GPS coordinates | Number of individuals | Dominance index between species of malaria mosquitoes, $f \pm s f$, % |  |
|-----|---------------------------------------------|----------------------------------|-----------------------|---------------------------------------------------------------------|---|
| 1   | Russia, Tomsk region, Kolarovo village      | 07.06.2017 56°32´85”N 84°94´80”E | 105                   | 96.2±1.9 3.8±1.9 0                                                  |   |
| 2   | Republic of Kazakhstan, Kokshetau           | 22.06.2017 53°18´17”N 69°19´17”E | 78                    | 100 0 0                                                           |   |
| 3   | Republic of Kazakhstan, Semey               | 24.08.2017 50°2503 ´N 80°1211”E | 246                   | 100 0 0                                                           |   |
| 4   | Republic of Kazakhstan, Urdschar district, Naualy village | 28.07.2017 46°59´45”N 81°45´31”E | 151                   | 49.6±4.1 0 50.4±4.1                                               |   |
| 5   | Republic of Kazakhstan, Urdschar district, Malak village | 13.08.2017 47°01´05”N 81°44´18”E | 163                   | 100 0 0                                                           |   |
| 6   | Republic of Kazakhstan, Urdschar district, Koldenen village | 31.07.2016 47°06´44”N 81°54´47”E | 145                   | 100 0 0                                                           |   |
| 7   | Republic of Kazakhstan, Urdschar district, Pervomaisk ponds | 05.08.2017 47°07´42”N 81°65´13”E | 104                   | 50.0±4.1 50.0±4.1                                               |   |
| 8   | Republic of Kazakhstan, Turksib district, Pervomaisk ponds | 16.07.2014 43°22´47”N 76°54´44”E | 121                   | 100 0 0                                                           |   |

In the *An. messeae* populations, the following genocomplexes are distinguished (in parentheses, areas of the range dominated by these chromosomal combinations):

1) XL$^{11}$2R$^{11}$3R$^{11}(01,00)3L$^{00}(01,11)$ (north);  
2) XL$^{22}(12)2R^{11}(01,00)3R^{11}(01,00)3L^{00}(01,11)$ (northeast);  
3) XL$^{11}2R^{01}3R^{00}(01,11)3L^{00}(01,11)$ (center);  
4) XL$^{00}(01)2R^{00}3R^{00}(01,11)3L^{00}(01)$ (southwest);  
5) XL$^{11}2R^{00}3R^{00}(01,11)3L^{00}(01,11)$ (southeast).

Theoretically, the *An. messeae* karyofound can be divided into 2 parts: the “northern” genocomplexes (1+2) and the “southern” genocomplexes (4+5). Genocomplexes (3) are formed when the imago crosses with the “northern” and “southern” karyotypes and are much rarer than expected based on the frequencies of the chromosomal variants that compose them [4].
**Chromosome XL.** The same southeastern variant of the genocomplex dominates in all *An. messeae* populations from Eastern Kazakhstan: XL₁ males, XL₁₁ females (Table 2), and inversions of the northeastern genocomplexes XL₂₂ and XL₁₂, which are also noted in populations from Western Siberia. These variants of inversions are not found in the southern regions of Eastern Kazakhstan (Pervomaisk ponds). In the south of the species habitat, there has always been a stable system – on the sex chromosome 100% dominance of XL₁₁ inversion [4, 20]. Since 2014, changes have been noted. Thus, in the sample of Pervomaisk ponds, the presence of individuals with chromosomal variants of the south-western genocomplex XL₀₀ and XL₀₁ was noted.

In the Kolarovo population from the south of Western Siberia, in contrast to the Kazakhstan populations, frequencies with inversions of the south-western genotype, XL₀₀ and XL₀₁, prevail [21]. By 2013, the frequency of occurrences of inversions XL₀₀ and XL₀₁ in populations from Kolarovo village increased from 1.0 ± 0.8% to 43.0 ± 5.9% and from 4.0 ± 1.5% to 35.0 ± 5.3%) [21]. The authors associate these changes with global warming.

**Chromosome 2R.** On the right shoulder of the second chromosome of *An. messeae* in the populations from Eastern Kazakhstan there are no “northern” variants with 2R₁₁ inversion; only in Semey city, a variant with 2R₀₁ inversion with a frequency of 3.4 ± 3.4% was detected. In all other populations, polymorphism was not detected, all karyotypes contained the evolutionary initial “southern” variant 2R₀₀.

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**Table 2. Chromosome structure of local populations of mosquito *An. messeae* in different regions of Eastern Kazakhstan**

| Chromosomal variants | Frequencies of chromosomal variants, f ± s f, % | Males, n | Females, n | Both sexes, n |
|----------------------|------------------------------------------------|---------|------------|--------------|
|                      | Semey | Kokshetau | Nauly | Malak | Aksakovka | Pervomaisk ponds |
| XL₀₁ | 6.9±4.7 | 0 | 3.9±3.8 | 0 | 8.3±5.6 | 0 |
| XL₁ | 27.6±8.3 | 36.4±10.3 | 26.9±8.7 | 30.0±8.4 | 20.9±8.3 | 38.5±13.5 |
| XL₂ | 0 | 4.5±4.4 | 11.5±9.3 | 3.3±3.3 | 12.5±6.7 | 0 |
| n | 10 | 9 | 11 | 10 | 10 | 5 |
| XL₀₀ | 3.4±3.4 | 0 | 7.7±5.2 | 0 | 8.3 | 7.7±7.4 |
| XL₀₁ | 3.4±3.4 | 4.5±4.4 | 0 | 0 | 0 | 0 |
| XL₁₁ | 27.8±8.3 | 41.0±10.5 | 34.6±9.3 | 63.4±8.8 | 25.0±8.8 | 53.8±13.8 |
| XL₁₂ | 20.8±7.5 | 0 | 0 | 0 | 0 | 0 |
| XL₂₂ | 3.4±3.4 | 13.6±7.3 | 15.4±7.1 | 3.3±3.3 | 25.0±8.8 | 0 |
| XL₁₃ | 6.9±4.7 | 0 | 0 | 0 | 0 | 0 |
| n | 19 | 13 | 15 | 20 | 14 | 8 |
| 2R₀₀ | 96.6±3.4 | 100 | 100 | 100 | 100 | 100 |
| 2R₀₁ | 3.4±3.4 | 0 | 0 | 0 | 0 | 0 |
| 2R₁₁ | 0 | 0 | 0 | 0 | 0 | 0 |
| 3R₀₀ | 6.9±5.2 | 31.8±9.9 | 7.7±5.4 | 43.3±9.0 | 45.8±10.1 | 69.2±8.4 |
| 3R₀₁ | 48.3±10.2 | 59.1±10.5 | 57.7±10.0 | 50.0±9.1 | 12.5±6.7 | 30.8±8.4 |
| 3R₁₁ | 44.8±10.1 | 9.1±6.1 | 34.6±9.7 | 6.7±4.5 | 41.7±10.0 | 0 |

The frequencies of all variants of the chromosome 2R inversion, presented in 1974 in populations from Kolarovo village: 2R₀₀ – 31.0 ± 3.6%, 2R₀₁ – 50.0 ± 3.8%, 2R₁₁ – 19.0 ± 3.1. In recent years, in this population, the 2R₀₀ inversion manifests itself at the level of 95.4 ± 2.3%, variants 2R₀₁ with a frequency of 3.5 ± 2.0%; the frequency of variant 2R₁₁ is 1.4 ± 1.3%. Thus, the replacement of the “northern” genocomplexes with the “southern” ones is observed [21]. In Kazakhstan populations of *An. messeae* in the process of global warming, apparently, the replacement of the south-eastern genocomplexes by the south-western karyotypes is observed.
Chromosome 3R. In all populations from Eastern Kazakhstan, all variants of inversions in 3R are observed, except for the population from Pervomaisk ponds, where there is no inversion of 3R_{11}. In Semey city, the maximum frequency of 3R_{11} homozygotes is observed. The lowest frequency of variants with inversion 3R_{00} is noted in Semey city with a frequency of 6.9 ± 5.2%. According to the inversion frequencies of 3R, the Kazakhstan and Siberian mosquitoes (Table 2). In the Kolarovo population, the inversion of 3R_{00} manifests itself at the level of 75.9 ± 4.6%, and the inversion of 3R_{01} – 8.0 ± 2.9% [21].

Chromosome 3L. In all populations from Eastern Kazakhstan, all variants of inversions in 3L are observed. The lowest frequency of variants with inversion 3L_{11} is noted in the village of Naualy, as well as inversion 3R_{11}. In Semey city, the maximum frequency of heterozygotes 3L_{01} is observed – 72.4±8.3%, in contrast to other populations. In terms of the inversion frequencies of 3L, as well as the inversions of 3R, Kazakhstan’s mosquitoes are clearly distinct from the Siberian ones. In the Kolarovo population, the inversions of 3L_{00} are manifested at the level of 80.5 ± 4.3%, and the inversions of the 3L_{11} – 0% [21]. In Eastern Kazakhstan populations, the inversion frequencies of 3L_{00} increase from north to south from 20.7% to 46.1%.

The karyotypic diversity (a variants combination of the gonosome and autosomes in the nuclei of cells in different individuals) in Eastern Kazakhstan decreases from north to south. The highest level of karyotypic diversity was found in samples from Semey city where, in aggregate, 16 karyotypes were found in males and females; and the minimal – from the southern samples of Pervomaisk ponds – 9 karyotypes. For the period 1941-2014 (74 years), the average monthly, average seasonal and annual air temperatures increased almost everywhere in Kazakhstan. The rate of increase in average annual air temperature is about 0.27 °C for every 10 years [22]. On the territory of the republic, the number of days with severe frosts (when the daily minimum temperature is below minus 20 °C, FDM 20 index) is decreasing everywhere by 1–3 days in 10 years, but a significant decrease (2–3 days / 10 years) is noted only at several weather stations of the West Kazakhstan region and in the north-east of the republic.

Such a situation is favorable for the survival of anopheles mosquitoes in winter diapause. However, an increase in temperature in the summer time reduces water areas, which is negative for the premaginal phases of development. Recently, the development of larvae of anopheles mosquitoes has been observed along with Culex pipiens species in artificial ponds and small tanks.

Thus, the population of anopheles mosquitoes in Western Siberia and Eastern Kazakhstan differ in species composition. However, the identified trends in the karyotypic composition of An.messeae mosquitoes in populations of Eastern Kazakhstan probably repeat the processes that have occurred in Western Siberia since the 80s and are associated with climate warming in the studied region.

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