

## Cytogenetic and environmental analysis of the populations of the *Anopheles messeae* mosquito of the eastern Kazakhstan region and in the foothills of Alatau

G M Abylkassymova<sup>1,3</sup>, A K Muminov<sup>2</sup>, V P Perevozkin<sup>2,3</sup>, A K Sibataev<sup>3</sup>,  
V N Stegnyy<sup>3</sup>

<sup>1</sup> Institute of General Genetics and Cytology of the National Academy of Sciences of the Republic of Kazakhstan, Almaty

<sup>2</sup> Tomsk State Pedagogical University, Tomsk

<sup>3</sup> Tomsk State University, Tomsk

E-mail: gulyam05@mail.ru

**Abstract** Cytogenetic analysis of populations of larvae of malarial mosquitoes of the East Kazakhstan region (Semey city, Urdzhar village) and in the foothills of Alatau. In the village of Urdzhar identified two species *Anopheles*: *An. claviger* and *An. messeae*; in the remaining studied areas - only *An. messeae*. In populations *An. messeae* established a high level of inverse polymorphism on the sex chromosome and the third autosome. Significant differences between alternative samples for the composition of inversions and the frequencies of their variants due to the ecological peculiarities of the regions were recorded. In the south of the species range (Almaty agglomeration) over the past decades, a significant change in the frequency of chromosome inversions associated with climate change has been revealed.

### Introduction

The study of the *Anopheles* mosquitoes fauna in Kazakhstan is a fundamental and applicative problem due to the fact of their epidemiological significance. The unique natural and climatic conditions on the most part of the territory of Kazakhstan set up the favorable environment for the *Anopheles* mosquitoes population growth. Starting in 1996 through 2011 as many as 118 cases of people getting malaria were registered in the City Clinical Infectious Disease Hospital of Almaty, 114 of them were introduced cases, 4 cases of unknown epidemiology [1]. Although the WHO report on malaria in 2012 had pointed out the status of Kazakhstan as the country free from malaria, due to the high population migration through the south-eastern regions of the country and the importation of infection from the countries of Central Asia, this region should be defined as the territory of a sustainable malaria risk.

Within Kazakhstan, the numerous studies on different aspects of this group of insects have been carried out using various methods [2-6]. One of the problems of studying the *Anopheles* mosquitoes in this region is the presence of sibling species and their just identifiability.

In recent years, molecular and cytogenetic methods start to get widely used and make it possible to accurately identify species in complex taxonomic groups of *Anopheles* mosquitoes and to conduct an analysis of intrapopulation genetic polymorphism in order to study the history and geographical variability of populations and hybridogenesis at the borders of allopatric populations of species.

One of the most taxonomically complex species is the sibling species of the *Anopheles maculipennis* complex (Diptera, Culicidae). In Kazakhstan, there are 3 species of this complex: *An.*



*messeae*, *An. martinius* and *An. artemievi*. Out of the *An. maculipennis* complex only *An. artemievi* species is found in the South Kazakhstan and Dzhambul regions [7, 8]. This species was discovered and described relatively recently with the help of molecular genetic methods. Chromosome-polymorphic *An. messeae* is the most prevailing species in Kazakhstan. This species is found everywhere except for Mangistau, Kyzylorda and South-Kazakhstan regions [5]. Cytogenetic analysis of Kazakhstan's anopheles mosquitoes was being conducted since the 1980s [6, 9-11].

The purpose of this work is to study the changes in the karyotypic structure of the *Anopheles* mosquitoes populations in the southern and eastern regions of the Republic of Kazakhstan.

### Materials and methods

The study subjects were anopheles mosquitos of the *An. messeae Falleroni* species, inhabiting the Eastern regions of Kazakhstan and in the foothills of Alatau. The presence of polytene chromosomes in the salivary gland cells of larvae offers the possibility of using the cytogenetic method to study inversion polymorphism in natural populations. The chromosomal rearrangements were used as genetic markers to assess the selective effect of various environmental factors on a population.

The material for the study was larvae of larval stage IV obtained from two districts of the East Kazakhstan region (Urdzhar village and Semei), and also from in the foothills of Alatau, their samples were generated in 2013-2014. The larvae were fixed in ethanol: glacial acetic acid, 1 : 3 (Carnoy's solution). Lactoaceto orcein preparations were prepared according to the modified method. Cytogenetic analysis of the inversions was carried out using the *An. messeae* chromosome maps.

### Results and discussion

These two studied alternative populations from the East Kazakhstan region significantly differ in inverse composition (Table 1).

**Table 1.** The inversion structure of the studied larval populations of *An. messeae* originated from the East Kazakhstan region and in the foothills of Alatau

Chromosome variants	Frequencies (%) of inversion genotypes in the biotope population		
	Village Urdzhar	City of Semei	City of Almaty
Males (gonosomes, hemizygotes)			
XL <sub>0</sub>	–	5,6	64
XL <sub>1</sub>	100,0	88,8	36
XL <sub>2</sub>	–	5,6	–
N	19	18	11
Female (gonosomes, zygotes)			
XL <sub>00</sub>	–	–	71
XL <sub>01</sub>	6,2	–	–
XL <sub>11</sub>	93,8	57,2	29
XL <sub>12</sub>	–	21,4	–
XL <sub>22</sub>	–	14,3	–
XL <sub>13</sub>	–	7,1	–
N	32	14	14
Both sexes (autosomes)			
2R <sub>00</sub>	100	96,9	100
2R <sub>01</sub>	–	3,1	–
3R <sub>00</sub>	21,6	12,6	20
3R <sub>01</sub>	56,8	43,7	28

Chromosome variants	Frequencies (%) of inversion genotypes in the biotope population		
	Village Urdzhar	City of Semey	City of Almaty
3R <sub>11</sub>	21,6	43,7	52
3L <sub>00</sub>	51,0	15,6	84
3L <sub>01</sub>	45,1	56,2	16
3L <sub>11</sub>	3,9	28,2	–
<i>n</i>	51	32	25
<i>n</i> , total number of individuals; <i>N</i> , the number of individuals with given chromosome.			

In both samples, the same variants of the gonosome are dominant: XL<sub>1</sub> in males, and XL<sub>11</sub> in females. At the same time, the diversity of sex chromosome inversions in the south of the region is much lower - only one more variant is found in the composition of XL<sub>01</sub> heterozygotes in females. Previously, the XL<sub>0</sub> inversion was not detected in these populations [6, 9, 10]. In the north of the region, in Semey, four inversion sequences of gonosomes have been found, both in homo- and heterozygotes, including the XL<sub>13</sub> inversion, rare occurrence for the region. In the same region, the 2R<sub>01</sub> inversion was found in the heterozygote by the second autosome of the right arm, the common case in the north of the Palaearctic, but not typical of Kazakhstan. The left arm of the second autosome, as might be expected, turned out to be monomorphic in both samples. By the nature of the occurrence of the third autosome's inversion variants, both arms demonstrate inverse frequency trends in two alternative samples. In the south of the region, in Urdzhar village, the evolutionarily original 3R<sub>00</sub> and 3R<sub>00</sub> homozygotes are relatively common, while in the north, in Semey, 3R<sub>11</sub> and 3L<sub>11</sub> are the significantly more common ones ( $p = 0.05$ ). Furthermore, the respective 3R<sub>01</sub> and 3L<sub>01</sub> heterozygotes ( $p = 0.05$ ) are statistically equally often-observed in the samples.

According to the karyotypic diversity criterion (the combination of variants of gonosomes and autosomes in the nuclei of cells of individual species), it is important to single out a sample from Semey where, in total, 16 karyotypes were registered in males and females; in the sample from Urdzhar village only 11 variants were recorded (Table 2).

**Table 2.** Karyotypic structure of the studied larval populations of *An. messeae* originated from the East Kazakhstan region

Chromosome variants	Frequencies (%) of inversion genotypes in the biotope population			
	Village Urdzhar		City of Semey	
	<i>N</i>	frequency (f ± m), %	<i>N</i>	frequency (f ± m), %
XL <sub>01</sub> 2R <sub>00</sub> 3R <sub>00</sub> 3L <sub>01</sub>	1	1,9±1,9	-	-
XL <sub>01</sub> 2R <sub>00</sub> 3R <sub>01</sub> 3L <sub>00</sub>	1	1,9±1,9	-	-
XL <sub>11(1)</sub> 2R <sub>00</sub> 3R <sub>00</sub> 3L <sub>01</sub>	7	13,7±4,8	3	9,3±5,1
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>01</sub> 3L <sub>01</sub>	8	15,7±5,1	5	16,6±6,6
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>01</sub> 3L <sub>00</sub>	18	35,4±6,7	2	6,2±4,3
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>01</sub>	6	11,8±4,5	3	9,3±5,1
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>00</sub>	4	7,8±3,7	3	9,3±5,1
XL <sub>11</sub> 2R <sub>00</sub> 3R <sub>00</sub> 3L <sub>00</sub>	3	5,9±3,3	-	-
XL <sub>11</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>01</sub>	1	1,9±1,9	1	3,1±3,1
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>11</sub>	1	1,9±1,9	2	6,2±4,3
XL <sub>11,1</sub> 2R <sub>00</sub> 3R <sub>01</sub> 3L <sub>11</sub>	1	1,9±1,9	4	14,5±6,2
XL <sub>12</sub> 2R <sub>00</sub> 3R <sub>01</sub> 3L <sub>01</sub>	-	-	2	6,2±4,3
XL <sub>12</sub> 2R <sub>00</sub> 3R <sub>00</sub> 3L <sub>11</sub>	-	-	1	3,1±3,1

Chromosome variants	Frequencies (%) of inversion genotypes in the biotope population			
	Village Urdzhar		City of Semey	
	<i>N</i>	frequency (f ± m), %	<i>N</i>	frequency (f ± m), %
XL <sub>0</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>01</sub>	-	-	1	3,1±3,1
XL <sub>22</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>11</sub>	-	-	1	3,1±3,1
XL <sub>2</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>01</sub>	-	-	1	3,1±3,1
XL <sub>1</sub> 2R <sub>01</sub> 3R <sub>01</sub> 3L <sub>01</sub>	-	-	1	3,1±3,1
XL <sub>22</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>01</sub>	-	-	1	3,1±3,1
XL <sub>13</sub> 2R <sub>00</sub> 3R <sub>11</sub> 3L <sub>11</sub>	-	-	1	3,1±3,1
<i>n</i>	51		32	

*n* – number of individuals; *N*, number of individuals with given chromosome inversion.

It is fair to say that the aforementioned differences in the inversion and karyotypic composition of the studied populations were set by different selection vectors in the differing environmental conditions of the north and south of the East Kazakhstan region. It is known that the temperature regime in the winter and spring seasons is a limiting factor for anopheles mosquitoes, and it also determines chromosomal diversity in *An. messeae* populations in the summer.

In average, in 1941-2014 throughout Kazakhstan the rate of increase in the average annual temperature was 0.27 °C per decade [12]. Seasonally, the highest temperature increase occurred in spring and autumn - 0.31 °C per decade, slightly lower rates were registered in winter - 0.27 °C per 10 years, and in the summer the slowest rate of temperature increase was observed - 0.19 °C per decade. As with other mosquito-borne diseases, climate warming can change the picture of malaria spreading on the globe [13].

The temperature regimes in the winter and spring periods for Semey and Urdzhar village in 2013-2014 are presented in Table 3. Average seasonal temperatures in Semey are lower than those in Urdzhar [14], but it should be noted that the daily temperature changes in the specified seasons averagely are much more pronounced in Urdzhar than in Semey. Such differences are also linked to the location of these biotopes at a certain altitude above sea level. Urdzhar is located in a more elevated area (512 m ASL), and Semey is in the steppe (196 m ASL). Thus, a smaller inversion and karyotypic diversity of mosquito populations in the south of the East Kazakhstan region is due to more extreme environmental conditions (daily temperature changes in higher altitude regions).

**Table 3.** Temperature regime in the settlements of the region for 2013-2014. [14]

Month	Average temperatures in settlements, °C									
	City of Semey					Village Urdzhar				
	Per month		During the season			Per month		During the season		
	day	night	day	night	twenty-four hours	day	night	day	night	twenty-four hours
<i>December</i>	-3,8	-6,8	-9,8	-12,9	-11,4	-0,9	-9,7	-5,8	-15,7	-10,8
<i>January</i>	-10,8	-13,9				-6	-17,7			
<i>February</i>	-14,9	-18,0				-10,6	-19,6			
<i>March</i>	2,4	-2,0	11,6	6,9	9,3	3,8	-5,1	14,2	6,7	10,5
<i>April</i>	12,9	8,7				15,5	8,4			
<i>May</i>	19,5	14,1				23,3	16,7			

It is possible that the established differences in the inversion structure of the studied populations are also associated with the competitive relations of the preimaginal stages of two mosquito species – *An. messeae* and *An. claviger*. In the Urdzhar population, unlike the Semey one, about 50% of population is of *An. claviger* species.

The larvae caught in the foothills of Alatau (1982) are homozygous by chromosomal sequences  $XL_1$  and  $2R_0$ , but polymorphous by inversion of chromosomes 3R and 3L (the frequencies of chromosome variants,  $f \pm sf$  (%):  $3R_{00}=9,9 \pm 3,5$ ;  $3R_{01}=52,1 \pm 5,9$ ;  $3R_{11}=38,0 \pm 5,8$ ;  $3L_{00}=43,7 \pm 5,9$ ;  $3L_{01}=42,2 \pm 5,9$ ;  $3L_{11}=14,1 \pm 4,1$ ) [9].

Our analysis of the chromosome composition of mosquitoes of this population, conducted in 2014, showed significant changes in last 32 years. For the first time mosquitoes with chromosomal variants of the sequence  $XL_0$ , ( $XL_0 = 64 \pm 15.2\%$  and  $XL_0 = 71 \pm 9.3\%$ ), absent in 1982, and, more so, at the present time a strong disruptive selection against  $XL_{01}$  heterozygotes is being observed. The concentration of  $3L_0$  version (from 60% in 1974 to 90% in 2014) has also increased, and the frequency of occurrence of  $3R_0$  decreased slightly (from 30% in 1974 to 20% in 2014). Almaty population of *An. messeae* species in 1982 was similar in composition to the population from the valley of the Chu river, however, it had an increased frequency of homozygotes of  $3L_{11}$ . In this population there have also been changes in the frequencies for these versions of the sequence, an increase in the frequencies of  $3L_{00}$  ( $3L_{00}=84,0 \pm 7,5$ ;  $3L_{01}=16,0 \pm 7,5$ ).

Thus, over the past decades in the south of area of *An. messeae* species a significant change in the frequency dynamics of chromosome inversions was revealed. Earlier similar microevolutionary processes were detected in the north of the *An. messeae* species area due to the changes in climatic factors [11].

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