

Chromosomal Polymorphism of Malaria Mosquitoes of Karelia and Expansion of Northern Boundaries of Species Ranges

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Abstract—Chromosomal variability in peripheral populations of malaria mosquitoes of the *Anopheles* genus (Diptera, Culicidae) inhabiting the territory of Karelia has been studied. The modern northern limits of the ranges inhabited by sibling species of malarial mosquitoes *An. beklemishevi*, *An. daciae*, *An. messeae* s. s. and *An. maculipennis* were established. After 2010, the boundary of distribution of malaria mosquitoes shifted northward by 170 km, from the 65th parallel to the Arctic Circle. Inversion heterozygotes XL₁, XL₂, 2R₂, 3R₁, and 3R₅ were found in peripheral populations of *An. beklemishevi*. Peripheral populations of *An. messeae* s. s. were homozygous for inversion of sex chromosome XL₁ and differed in the frequencies of autosome inversions from the middle taiga populations. The frequency of heterozygotes for autosomal inversions 2R₁, 3R₁, and 3L₁ increased in populations at the edge of the species range. Chromosomal variability in peripheral populations contributes to the dispersal of malaria mosquitoes in high latitudes under warming climate conditions.

Keywords: chromosomal polymorphism, marginal populations, boundaries of ranges, malaria mosquitoes, *Anopheles*

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INTRODUCTION

The macroevolutionary processes in populations living at boundaries of their range influence species adaptation and allow the species expand to new territories. Populations located on the periphery of the species ranges have been recognized as having little value in conserving biodiversity. Peripheral populations experience genetic and ecological structure variation, significant fluctuations in population parameters, population isolation, reduction in gene exchange, and increased genetic differentiation [1]. The number and genetic structure of peripheral populations are strongly dependent on the effects of limiting factors that restrict the ability of a population to survive at the edges of the species range. Ambient temperature is an important determinant of transmission of malaria mosquitoes from the *Anopheles* genus (Diptera, Culicidae) inhabiting the Northwest European Russia, including the area of Fennoscandia. Favorable ocean climate has enhanced proliferation of the malaria-carrying mosquitoes at higher altitudes. In the middle of the 20th century, the northern boundary of malaria transmission ran south of the 65th parallel [2]. Climate change allows a northward spread of some species of malaria-carrying mosquitos, with chromo-

somal changes in the populations inhabiting the northern taiga zone of Eurasia. The purpose of this work was to determine the current boundaries of the ranges and chromosomal polymorphism in peripheral populations of closely related species of malaria mosquitoes in Karelia.

MATERIALS AND METHODS

Malaria mosquitoes were collected as the fourth instar mosquito larvae from nine mosquito breeding sites in Karelia in 2009–2010 and 2022. Larval instars were collected with a medical cuvette from the surface of the water. Each individual mosquito from the 2022 samples was divided into two parts. The head and thorax of the larva were fixed in Clarke's fluid (three parts of 95% ethanol and one part of glacial acetic acid) and used for cytogenetic analysis. The larva's abdomen was fixed in 95% ethanol for molecular genetic studies. All specimens were stored in individual tubes at –20°C.

The polytene chromosomes were prepared from the salivary glands of third and fourth instar larvae. Paired salivary glands were dissected from the thorax in a fixing fluid. The glands were stained with 2% lacto-aceto-orcein for 60 min and washed in 45% ace-

Table 1. Primers for the identification of *An. messeae*/*An. daciae* sibling species using the ITS1 region of ribosomal DNA cluster

No.	Primer	Primer sequence 5'–3'	Length of PCR fragment, bp	Polymorphic sites in reference sequence from GenBank ID AY648982 used for identification of <i>An. messeae</i> / <i>An. daciae</i>	
				412	432
1	ITS638F	TGAACTGCAGGACACATGAAC	471	G/A	G/C
2	ITS638R	CCTACGTGCTGAGCTTCTCC			

tic acid solution. After washing, the glands were squashed with glass coverslips. The resulting specimens were assessed using a Nikon Eclipse E200 light microscope. Sibling species *An. beklemishevi* Stegny et Kabanova, 1976, *An. maculipennis* Meigen, 1818, and *An. messeae* Falleroni, 1926 were differentiated according to the banding patterns of polytene chromosomes and comparison with the karyotype images of these species [3–5]. Homozygotes and heterozygotes were identified from chromosomal inversions in polymorphic species *An. beklemishevi* and *An. messeae* s. l. Karyotypes were studied in 1595 mosquitoes. The interpopulation variability in the frequency of inversion genotypes was assessed using the chi-square (χ^2) test [6]. The standard fixation index F_{ST} was estimated using the Fstat 2.9.4 software package [7].

Molecular identification was performed using isolated larval abdomens. Each single larva was analyzed individually. Total DNA was isolated by phenol chloroform extraction. The concentration of DNA was measured in an Implen NanoPhotometer NP80 spectrophotometer. The concentration was adjusted to 30–60 ng/ μ L. PCR was run in a final volume of 20 μ L using the EncycloPlus PCR amplification kit (Eurogen, Russia) as recommended by the manufacturer. ITS2 fragments were obtained using primers designed in the Primer3 software (<https://primer3.ut.ee/>). The primers are characterized in Table 1.

PCR products were stained with ethidium bromide and electrophoresed in a 1.5% agarose gel and TBE buffer. Identification of *An. messeae*/*An. daciae* species by restriction fragment length polymorphism (RFLP) PCR was performed using *RsaI* endonuclease (SibEnzyme, Russia). *An. daciae* and *An. messeae* have a different number of restriction sites for *RsaI* endonuclease (3 and 4, respectively). The length of restriction fragments was 10, 47, 50, and 364 bp for *An. daciae* and 10, 47, 50, 72, and 292 bp for *An. messeae*. To confirm RFLP identification, random specimens from the two northern mosquito habitats in the village of Chupa and the town of Kem were subjected to Sanger sequencing. For sequencing, DNA was purified of gel using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, United States) in accordance with the manufacturer's instructions. The nucleotide sequence of PCR fragments amplified

using forward and reverse primers was determined using a 3500 Genetic Analyzer and BigDye® Terminator v3.1 Cycle Sequencing Kit reagents (Applied Biosystems, United States) according to the manufacturer's recommendations.

Bioinformatic analysis of chromatograms was performed using the ChromasPro 13.3 software (Technelysium, Australia). The sequences obtained by sequencing were aligned to the sequences deposited in GenBank database using NCBI resources (<http://www.ncbi.nlm.nih.gov>).

RESULTS AND DISCUSSION

Species Composition and Range Boundaries

The fauna of malarial mosquitoes in Karelia includes closely related sibling species of the *Maculipennis* complex: *An. beklemishevi*, *An. maculipennis* s. s., and *An. messeae* s. l. (the latter includes the *An. daciae* Linton, Nicolescu & Harbach, 2004 and *An. messeae* s. s. sibling species) (Table 2). The species of malaria mosquitoes are unequally distributed on the territory of Karelia (Fig. 1). All the species inhabit the same habitats in the south and in the middle taiga subzone. The conditional boundary of the middle taiga in Karelia runs near the 63rd parallel [8]. *An. maculipennis* and *An. messeae* s. l. are ubiquitous in larval biotopes south of the 63rd parallel, whereas mosquitoes *An. beklemishevi* have a low frequency of occurrence (1.1–7.2%) and do not inhabit all water bodies. A different territorial distribution of species is observed north of the 63rd parallel, in the northern taiga subzone. All of the listed species are found in biotopes up to the 65th parallel. The proportion of *An. beklemishevi* in common breeding sites reaches 16.0–64.6%. Apparently, the 65th parallel served as the northern boundary for the spread of malaria mosquitoes in Karelia in 2010 [9]. Larvae of malaria mosquitoes were not found in 2022 in several surveyed water bodies in the vicinity of Loukhi urban-type settlement (66.077640° N, 33.075553° E). One of the tasks of field research in 2022 was to determine the modern northern boundaries of the habitats of sibling species. The local population of *An. beklemishevi* is found in Lake Nigrozero (66.551536° N) near the Arctic Circle (66.5622° N). Over 12 years, the boundaries of the range of malaria-carrying mosquitoes have

Table 2. Species composition of malaria mosquitoes in Karelian habitats

No.	Habitat (coordinates)	Date of collection	Number of individuals	Dominance index ($f \pm s_f$, %)		
				<i>An. messeae</i> s. l.	<i>An. maculipennis</i>	<i>An. beklemishevi</i>
1	Petrozavodsk, drainage channel in floodplain of the Rybka River (61.795465° N, 34.280368° E)	August 2, 2022	143	0.7 ± 0.7	99.3 ± 0.7	0
2	Petrozavodsk, swampy floodplain of the Lososinka River (61.777579° N, 34.354456° E)	July 23, 2009	110	28.2 ± 4.3	71.8 ± 4.3	0
3	Petrozavodsk, swampy region (61.779865° N, 34.363274° E)	August 10, 2010	271	40.6 ± 3.0	58.3 ± 3.0	1.1 ± 0.6
4	Prionezhsky district, Shuya urban locality, swampy floodplain of the Shuya River (61.890139° N, 34.248805° E)	July 23, 2009	46	100	0	0
5	Kondopoga, pond (62.202047° N, 34.234599° E)	August 12, 2010	143	69.9 ± 3.8	27.3 ± 3.7	2.8 ± 1.4
6	Medvezhyegorsk, pond (62.918425° N, 34.451519° E)	August 13, 2010	169	47.9 ± 3.8	45.0 ± 3.8	7.1 ± 2.0
7	Segezha, drain around swamp (63.754374° N, 34.305334° E)	August 16, 2010	144	34.0 ± 3.9	1.4 ± 1.0	64.6 ± 4.0
8	Belomorsk, pond (64.512771° N, 34.778731° E)	August 15, 2010	190	75.8 ± 3.1	0	24.2 ± 3.1
9	Belomorsk, pond (64.544193° N, 34.787569° E)	July 31, 2022	51	96.1 ± 2.7	3.9 ± 2.7	0
10	Kem, Pueta River (64.951902° N, 34.565763° E)	August 14, 2010	137	1.5 ± 1.0	82.5 ± 3.2	16.0 ± 3.1
11	Kem, Pueta River (64.953534° N, 34.573063° E)	July 27, 2022	103	45.6 ± 4.9	54.4 ± 4.9	0
12	Loukhsky district, Chupa rural settlement, pond (66.261565° N, 33.021291° E)	July 28, 2022	42	100	0	0
13	Loukhsky district, Malinovaya Varakka rural settlement, Lake Nigrozero (66.551536° N, 32.757391° E)	July 20, 2022	46	0	0	100

shifted to the north by 170 km. The northernmost population of *An. messeae* was found 30 km to the south, in a water body in the village of Chupa, Loukhsky district (66.261565° N). The noted biotopes are the northernmost breeding sites for malaria mosquitoes in the European part of Russia. It is noted that larval habitats of the two species in the northern taiga are fragmentary. Many biotopes potentially suitable

for larval breeding are not occupied by malaria mosquitoes. For example, larvae and pupae were not found in more southern biotopes, i.e., in Lake Raudulambi, Ambarny rural settlement in the Republic of Karelia (65.925928° N, 33.117315° E).

In July 2022, we examined water bodies in Murmansk oblast located north of the Arctic Circle: near the town of Kandalaksha—a lake near the Lupche Bay

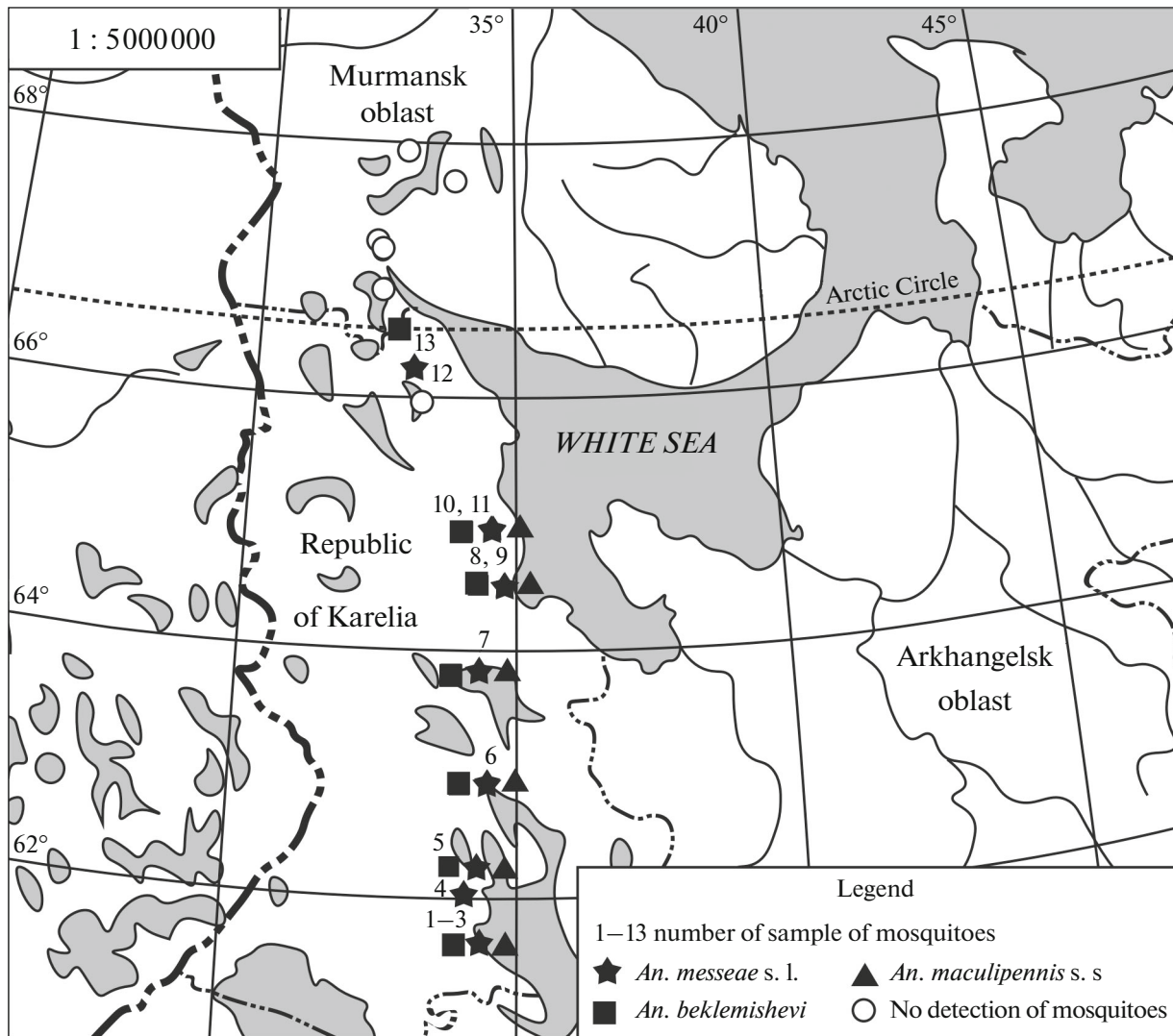


Fig. 1. The geographical distribution of malaria mosquito sibling species from the *Anopheles* genus in biotopes of Karelia. The coordinates and numerical designations of biotopes are given in Table 2.

(67.159583° N, 32.375519° E), a pond near the Lupche Bay (67.162094° N, 32.374103° E), the Niva River (67.147768° N, 32.424096° E), a pond near the Niva River (67.147184° N, 32.428109° E), a swamp near the Niva River (67.146078° N, 32.426983° E); a lake near the urban locality Zelenoborsky (66.866782° N, 32.390384° E); a lake in near the rural locality Knyazhaya Guba (66.869059° N, 32.399852° E); ponds in Monchegorsk (67.941813° N, 32.896272° E; 67.947667° N, 32.874325° E); swampy regions near the town of Kirovsk (67.648564° N, 33.702231° E). Larvae and pupae of malaria mosquitoes were found in none of these biotopes, and no newly emerged imago mosquitoes of the Maculipennis complex were detected. According to our data, mosquitoes of the *Anopheles* genus do not live within the northern taiga subzone of the Arctic circle (Kandalaksha, Zeleno-

borsky, Knyazhaya Guba, Kirovsk) and the Arctic forest tundra (Monchegorsk).

The northernmost habitat of *An. maculipennis* mosquitoes was detected in the town of Kem (64.953534° N). In the 1970s, the northern boundary of the species range ran through the Leningrad oblast, significantly south of Petrozavodsk [10]. Currently, this species has expanded its range to the northern taiga subzone of Karelia. *An. maculipennis* mosquitoes dominate in permanent and temporary larval biotopes of Petrozavodsk and Kem. In the 2022 sample from Petrozavodsk (temporary water body in the floodplain of the Rybka River), the proportion of *An. maculipennis* was 99.3%. Larvae of this species were found in biotopes located in the towns of Kondopoga, Medvezhyegorsk, Segezha and Belomorsk (Table 2). It appears that the warming of the climate resulted in the expansion of the range of *An. maculipennis* north-

wards. Warming climate in the taiga zone of Eurasia contributes to milder wintering conditions and a longer summer breeding season for malaria mosquitoes [11]. *An. maculipennis* mosquitoes not only occur in the north, but they also migrate eastwards, from the Volga region to the Southern Urals [12].

Chromosomal Polymorphism

The chromosomal variability in sibling species of malaria mosquitoes is associated with certain landscape-climatic zones [13]. It is observed in the populations of *An. beklemishevi* and *An. messeae* s. l., living on the territory of Karelia. Heterozygotes were found in populations of *An. beklemishevi* in the northern taiga subzone according to the following chromosomal inversions (the localization of inversions is indicated in parentheses): XL_1 (1d-3b), XL_2 (2c-5c), $2R_2$ (7c-9a), $3R_1$ (23b-26a), and $3R_5$ (27b-29c) [4]. The heterozygote frequencies by sex chromosomes were calculated in females (hereinafter, n is the number of studied individuals). The proportion of XL_{01} heterozygotes in Segezha was $2.0 \pm 2.0\%$ ($n = 50$) and in Belomorsk it was $15.4 \pm 7.1\%$ ($n = 26$). The proportion of XL_{02} heterozygotes was $20.0 \pm 5.7\%$ ($n = 50$) in Segezha, $7.7 \pm 5.2\%$ ($n = 26$) in Belomorsk, and $3.8 \pm 3.7\%$ ($n = 26$) in Nigrozero. Heterozygotes by autosomal inversions $3R_1$, $2R_2$, and $3R_5$ were found in the two northernmost populations of *An. beklemishevi*. The frequency of $3R_{01}$ heterozygotes was $2.2 \pm 2.2\%$ in Nigrozero ($n = 46$) and $6.5 \pm 3.6\%$ in Belomorsk ($n = 46$). Heterozygotes $2R_{02}$ and $3R_{05}$ were also detected in Belomorsk in 2010, each with the frequency of $2.2 \pm 2.2\%$ ($n = 46$). It is noted that chromosomal variability in *An. beklemishevi* remains at a low level in the Nigrozero population at the very edge of the species range.

A high level of chromosomal polymorphism was revealed for populations of *An. messeae* s. l. (Tables 3, 4). Homo- and heterozygotes were found in the karyotypes of *An. messeae* s. l. mosquitoes according to the following chromosomal inversions: XL_0 (2a-5b), XL_1 (accepted as standard), XL_2 (1b-4b), $2R_1$ (7b/c-12c/13a), $3R_1$ (23c/24a-26c/27a), $3L_1$ (34b/34c-37a/37b-38c/39a-39c/d) [5]. The $3L_1$ inversion originates from two overlapping inversions. The inversion frequencies vary in local populations of Karelia; however, in general, karyotypic diversity is typical of populations of *An. messeae* s. l. living in the taiga zone of Eurasia [13, 14]. The 2010 samples were used to compare the chromosomal composition of individuals from populations in the southern, central, and northern parts of Karelia [9]. In this work, we studied additional collections from 2009–2010 and compared the combined data on mosquito karyotypes from the middle taiga subzone (Petrozavodsk–Shuya–Kondopoga–Medvezhegorsk) and the northern taiga subzone (Belomorsk–Kem). It was found that mosqui-

toes in the middle and northern taiga differ in the genotype frequencies of the sex chromosome in females: in the northern taiga, the proportion of heterozygotes XL_{01} was higher ($\chi^2 = 9.77$; the number of degrees of freedom $d.f. = 1$; $p < 0.01$). Significant differences were found in the composition of autosomes. In the northern taiga, the proportion of homo- and heterozygotes by inversion $2R_1$ is higher ($\chi^2 = 7.57$; $d.f. = 2$; $p < 0.05$), but the frequency of heterozygotes with inversion $3L_1$ is lower ($\chi^2 = 7.76$; $d.f. = 1$; $p < 0.01$). We explain these differences by the different ratio of sibling species *An. daciae* and *An. messeae* s. l. in taiga biotopes. Mosquitoes of these species that belong to the *An. messeae* s. l. group carry several diagnostic inversions. Karyotypes with XL_0 inversion are hardly found in *An. messeae* s. s., while homo- and heterozygotes with $2R_1$ inversion are extremely rare in *An. daciae*. Chromosomal rearrangements $3R_1$ and $3L_1$ occur with different frequencies in both species [14]. Cases of interspecific mating in sympatric zones have been observed, but the proportion of such hybrids in taiga habitats is insignificant. It appears that hybrid species exhibit low adaptation, particularly in the habitats of the northern taiga zone.

The cytogenetic analyses of peripheral populations in the 2022 samples lead to intriguing data. The standard F_{ST} index reflects the level of interpopulation differences in chromosome composition (Table 5). Variability in the composition of sex chromosomes was evaluated separately for females, since males are hemizygous because they have only one polytenized sex chromosome XL. Each 2022 sample from the habitats of the northern taiga (Belomorsk, Kem, Chupa) significantly differs in F_{ST} value from any samples collected in the biotopes of the middle taiga, with the exception of the population in the village of Shuya in the south of Karelia (Table 5). Mosquitoes from the biotopes of the northern taiga are completely homozygous for inversion XL_1 , while *An. daciae* mosquitoes with inversion XL_0 are found in all larval biotopes of the middle taiga. It is important to note that females from the biotopes of Belomorsk in 2010 and 2022 had different composition with respect to sex chromosomes ($\chi^2 = 15.76$; $d.f. = 1$; $p < 0.001$). In 2022, no XL_{00} homozygotes or XL_{01} heterozygotes were found. Herein, it appears that the northern limits of distribution of *An. daciae* mosquitoes with XL_0 inversion are mobile and unstable. It is known that summer months of 2010 were abnormally hot on the Russian Plain and the north of the European Russia. The weather anomaly might have contributed to a temporary northward shift of the *An. daciae* range boundary. In particular, one female with the XL_{01} genotype was found in the larval biotope in Kem in 2010. There were no homo- and heterozygotes with XL_0 inversion in the 2022 sample in Kem. To clarify the species status of mosquitoes in the 2022 samples, a molecular genetic analysis was

Table 3. The frequencies of chromosomal variants in *An. messeae* populations in the middle taiga subzone in Karelia

Inversion homo- and heterozygotes	Frequencies of chromosomal variants, $f \pm s_f$, %				
	Petrozavodsk July 23, 2009	Petrozavodsk August 10, 2010	Shuya July 27, 2009	Kondopoga August 18, 2010	Medvezhyegorsk August 13, 2010
Males, <i>n</i>	63	14	17	39	34
XL ₀	34.9 ± 6.0	50.0 ± 13.4	41.2 ± 11.9	56.4 ± 7.9	76.5 ± 7.3
XL ₁	65.1 ± 6.0	50.0 ± 13.4	58.8 ± 11.9	43.6 ± 7.9	23.5 ± 7.3
Females, <i>n</i>	47	17	29	61	47
XL ₀₀	36.2 ± 7.0	58.8 ± 11.9	24.1 ± 7.9	37.7 ± 6.2	51.1 ± 7.3
XL ₀₁	14.9 ± 5.2	11.8 ± 7.8	13.8 ± 6.4	14.8 ± 4.5	12.8 ± 4.9
XL ₁₁	48.9 ± 7.3	29.4 ± 11.1	62.1 ± 9.0	47.5 ± 6.4	34.0 ± 6.9
XL ₁₂	0	0	0	0	2.1 ± 2.1
Both sexes, <i>n</i>	110	31	46	100	81
2R ₀₀	60.0 ± 4.7	71.0 ± 8.2	82.6 ± 5.6	76.0 ± 4.3	77.8 ± 4.6
2R ₀₁	39.1 ± 4.7	25.8 ± 7.9	10.9 ± 4.6	22.0 ± 4.1	19.7 ± 4.4
2R ₁₁	0.9 ± 0.9	3.2 ± 3.2	6.5 ± 3.6	2.0 ± 1.4	2.5 ± 1.7
3R ₀₀	52.7 ± 4.8	71.0 ± 8.2	39.1 ± 7.2	59.0 ± 4.9	67.9 ± 5.2
3R ₀₁	35.5 ± 4.6	19.3 ± 7.1	50.0 ± 7.4	33.0 ± 4.7	29.6 ± 5.1
3R ₁₁	11.8 ± 3.1	9.7 ± 5.3	10.9 ± 4.6	7.0 ± 2.6	2.5 ± 1.7
3R ₀₂	0	0	0	1.0 ± 1.0	0
3L ₀₀	90.0 ± 2.9	96.8 ± 3.2	93.5 ± 3.6	90.0 ± 3.0	96.3 ± 2.1
3L ₀₁	10.0 ± 2.9	3.2 ± 3.2	4.3 ± 3.0	10.0 ± 3.0	3.7 ± 2.1
3L ₁₁	0	0	2.2 ± 2.2	0	0

Table 4. The frequencies of chromosomal variants in populations of *An. messeae* in the northern taiga subzone in Karelia

Inversion homo- and heterozygotes	Frequencies of chromosomal variants, $f \pm s_f$, %				
	Segezha August 16, 2010	Belomorsk August 15, 2010	Belomorsk July 31, 2022	Kem July 27, 2022	Chupa July 28, 2022
Males, <i>n</i>	28	68	25	30	19
XL ₀	67.9 ± 8.8	47.1 ± 6.1	0	0	0
XL ₁	32.1 ± 8.8	52.9 ± 6.1	100	100	100
Females, <i>n</i>	21	76	26	17	23
XL ₀₀	23.8 ± 9.3	28.9 ± 5.2	0	0	0
XL ₀₁	52.4 ± 11.0	23.7 ± 4.9	0	0	0
XL ₁₁	23.8 ± 9.3	47.4 ± 5.7	100	100	100
Both sexes, <i>n</i>	49	144	49	47	42
XL ₀	57.1 ± 5.9	42.7 ± 3.3	0	0	0
XL ₁	42.9 ± 5.9	57.3 ± 3.3	100	100	100
2R ₀₀	71.4 ± 6.5	68.1 ± 3.9	57.2 ± 7.1	46.8 ± 7.3	16.7 ± 5.8
2R ₀₁	22.5 ± 6.0	23.6 ± 3.5	36.7 ± 6.9	44.7 ± 7.3	66.6 ± 7.3
2R ₁₁	4.1 ± 2.8	8.3 ± 2.3	6.1 ± 3.4	8.5 ± 4.1	16.7 ± 5.8
2R ₀₃	2.0 ± 2.0	0	0	0	0
3R ₀₀	44.9 ± 7.1	59.7 ± 4.1	46.9 ± 7.1	48.9 ± 7.3	47.6 ± 7.7
3R ₀₁	44.9 ± 7.1	34.7 ± 4.0	42.9 ± 7.1	38.3 ± 7.1	52.4 ± 7.7
3R ₁₁	10.2 ± 4.3	5.6 ± 1.9	10.2 ± 4.3	12.8 ± 4.9	0
3L ₀₀	100	97.9 ± 1.2	91.8 ± 3.9	87.2 ± 4.9	57.1 ± 7.6
3L ₀₁	0	2.1 ± 1.2	8.2 ± 3.9	8.5 ± 4.1	42.9 ± 7.6
3L ₁₁	0	0	0	4.3 ± 2.9	0

Table 5. F_{ST} values in pairwise comparisons of Karelian population samples by inversion variants of sex chromosomes in females and by autosomes in individuals of both sexes

Pairwise comparisons of samples by female sex chromosomes										
Samples	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Petrozavodsk 2010	Chupa 2022
Petrozavodsk 2009	0.0524	0.1728	0.0431	-0.0245	0.0084	0.0822	0.6745*	0.6278*	0.6686*	
Petrozavodsk 2010		0.0094	-0.0159	0.0192	-0.0177	-0.0122	0.3521*	0.3236*	0.3483*	
Shuya 2009			0.0189	0.1063	0.0461	0.0015	0.2679	0.2333	0.2633	
Kondopoga 2010				0.0139	-0.0199	-0.0082	0.3488*	0.3249*	0.3456*	
Medvezhyegorsk 2010					-0.0150	0.0403	0.4811*	0.4516*	0.4772*	
Segezha 2010						-0.0040	0.5065*	0.4610*	0.5005*	
Belomorsk 2010							0.2985*	0.2792*	0.2959*	
Belomorsk 2022								-	-	
Kem 2022										-
Pairwise comparisons of samples by autosomes in individuals of both sexes										
Petrozavodsk 2009	0.0036	0.0318	-0.0058	-0.0078	0.0215	-0.0086	0.0108	0.0281	0.1259*	
Petrozavodsk 2010		0.0095	0.0063	0.0256	0.0188	0.0036	-0.0049	0.0063	0.1026*	
Shuya 2009			0.0188	0.0572	0.0016	0.0335	0.0122	0.0324	0.1519*	
Kondopoga 2010				0.0031	0.0090	0.0051	0.0172	0.0395	0.1567	
Medvezhyegorsk 2010					0.0313	0.0091	0.0430	0.0695*	0.1897*	
Segezha 2010						0.0270	0.0315	0.0637*	0.2024*	
Belomorsk 2010							0.0064	0.0241	0.1330*	
Belomorsk 2022								-0.0047	0.0778	
Kem 2022									0.0382	

Asterisk indicates significant differences ($p < 0.05$).

performed. Sibling species *An. daciae* and *An. messeae* s. s. were identified by RFLP PCR. Ten specimens from the village of Chupa (sample no. 12) and 22 specimens from the town of Kem (sample no. 11) were studied. All specimens were identified as *An. messeae* s. s. To confirm the RFLP identification, PCR products were sequenced. All specimens had a common haplotype based on the ITS2 transcribed spacer region characteristic of *An. messeae* s. s. (GenBank ID: PP115571, PP115572). The results of cytogenetic and molecular genetic analysis indicate that *An. daciae* are absent in the biotopes of the northern taiga subzone. Evidently, the modern northern boundary of the range of *An. daciae* runs south of the 64th parallel, on the border of the middle and northern taiga.

The 2022 mosquito samples from the northern taiga habitats differed in the composition of autosomes from the larval samples from the biotopes in the middle taiga. Significant F_{ST} values were obtained by pairwise comparisons of samples from the northern mosquito populations of Kem and the village of Chupa with samples of mosquitoes from majority of other breeding sites (Table 5). The peripheral population from the village of Chupa is specific. Mosquitoes of this population differ from the combined sample from the northern biotopes of Kem and Belomorsk in an increased frequency of inversion heterozygotes $2R_{01}$ ($\chi^2 = 15.48$; $d.f. = 2$; $p < 0.001$), $3R_{01}$ ($\chi^2 = 6.60$; $d.f. = 2$; $p < 0.05$), and $3L_{01}$ ($\chi^2 = 17.05$; $d.f. = 1$; $p < 0.001$).

Therefore, we have identified the modern northern boundaries of the habitats of sibling species of malaria mosquitoes *An. beklemishevi*, *An. daciae*, *An. messeae* s. s., and *An. maculipennis* living in the taiga biotopes of Karelia. *An. beklemishevi* and *An. messeae* mosquitoes migrate northwards. The boundary of distribution of malaria mosquitoes has shifted from the 65th parallel to the Arctic Circle. The chromosomal composition in the peripheral populations of polymorphic species *An. beklemishevi* and *An. messeae* s. s. includes inversions typical of this landscape and climatic zone. Chromosomal rearrangements in marginal populations occur mainly in heterozygotes. Stabilizing selection is known to favor heterozygotes by such inversions and preserves the genetic variability at the periphery of the species range. Chromosomal rearrangements in peripheral populations are a basis for microevolutionary processes at the edges of species ranges and may contribute to subsequent high-latitude migration of mosquitoes in warming climate. This study has shown that the processes of adaptive radiation in malaria mosquitoes occur only in forest communities and do not run beyond the taiga zone.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of the Federal State University of Education, protocol no. 2 dated October 26, 2023.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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