Estimated resistance of the malaria mosquito *Anopheles messeae s.l.* to the insecticide malathion

Oleg. V. Vaulin✉, Dmitry. A. Karagodin, Elina. M. Baricheva, and Ilya. K. Zakharov

Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia, Oleg.v.vaulin@mail.ru

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ABSTRACT: Resistance to agricultural pesticides is an important and insufficiently studied concern for pest and disease vector research. We determined the malathion resistance of species in the *Anopheles maculipennis* mosquito group in a habitat near Novosibirsk, Russia. Most of the 851 individuals we measured were members of the *Anopheles messeae* s.l. complex (*An. messeae* and *An. daciae* species). The LC_{50} value for malathion was 0.052 mg/L for the mixed specimens, and we failed to find any differences between species. The LC_{50} value was within the range of values for malathion resistance of *Anopheles stephensi* and *Culex quinquefasciatus*. As the main resistance mechanism to organophosphate and carbamate insecticides is a single mononucleotide substitution in the *ace-1* gene, we searched for this mutation in *An. messeae* s.l. and *An. beklemishevi* by restriction analysis. This mutation was not found in 347 of the specimens. We sequenced the *ace-1* gene fragment for 24 specimens from four species of the *Anopheles maculipennis* group, including *An. messeae, An. daciae, An. atroparvus,* and *An. beklemishevi*. These specimens harbored a nucleotide substitution in the triplet where a mutation can lead to insecticide resistance, but this substitution would make it difficult for the resistance to develop. Since the studied specimens belong to branches of the Palearctic portion of the *Anopheles maculipennis* group, we suspect that all other Palearctic species of this group would have difficulties harboring the *ace-1* mutation that would lead to organophosphate and carbamate resistance. ***Journal of Vector Ecology* 44 (1): 48-56. 2019.**

Keyword Index: *Anopheles messeae* s.l., malathion, insecticide resistance, LC_{50}, *ace-1* gene, nucleotide substitution.

INTRODUCTION

The first successes in controlling insect pests were accompanied by the spread of mutations in populations that were associated with resistance to the insecticides. This spread made it necessary to emply insecticides from new chemical classes, but resistance to these new insecticides often developed as well. For example, a few years after the commencement of large-scale usage of DDT, insects resistant to DDT were reported (Denholm et al. 2002). Similarly, neonicotinoid-resistant species were detected approximately five years after administering this insecticide in practice. Inheritable mechanisms of insecticide resistance are mainly monogenic (ffrench-Constant 2013). Resistance to organophosphates and carbamates is usually caused by a single mutation in the acetylcholinesterase gene *ace-1* (ffrench-Constant 2013). This mutation, designated G119S, appears several times in different phylogenetic branches of mosquitoes in the genera *Culex* and *Anopheles* (Weill et al. 2004). A similar mutation was found in the homologous esterase gene of the fly, *Musca domestica* (ffrench-Constant 2006).

Insects may be resistant to insecticides even before the toxicants are used against them. For example, the carrion fly *Lucilia cuprina* carried an *esterase-3* variant that is associated with organophosphates resistance even before the insecticides were used (ffrench-Constant 2007). A mutation in the *Drosophila melanogaster Cyp6g1* gene, which is associated with resistance to many kinds of xenobiotics, arose a short time ago (Catania et al. 2004). This now abundant mutation was not found in *Drosophila* stocks that were founded before insecticide usage began (Daborn et al. 2002).

The most effective methods for mosquito control are the use of insecticide-treated nets and indoor treatment with residual insecticides (Chanda 2018). These nets also significantly decrease cases of mosquito-borne disease (Kleinschmidt et al. 2018). However, these methods are much less effective against the many mosquito vectors that are not strictly associated with the human environment, such as many *Anopheles* species including important the malaria vectors in the *Anopheles sinensis* complex and *An. arabiensis* (Sinka 2013). Since adult mosquitoes may be dispersed in large areas, and larval habitats are strictly associated with bodies of water, larval control is often more rational (Killeen et al. 2002). However, treating water bodies with insecticides could lead to inadvertent toxicity to non-target organisms.

The *Anopheles maculipennis* group, originating in North America with several branches, is epidemiologically important. One branch is Palearctic, in northern Africa and Eurasia north of the Himalayas (Kitzmiller et al. 1967, Vaulin and Novikov 2016) with ten species in the group, with *Anopheles messeae* s.l. as an interesting component. Two species, *An. messeae* A and B, were placed under a single species name, *An. messeae* (Novikov and Shevchenko 2001). These species were rediscovered in 2004 by the standard binomial names *An. daciae* and *An. messeae*, respectively (Nicolescu et al. 2004). *An. messeae* s.l. occupies a very large habitat area (Novikov and Shevchenko 2001, Novikov et al. 2004, Kronefeld et al. 2012). *An. messeae* s.l. species are predominant in a broad area of northern Eurasia (Novikov and Vaulin 2014, Novikov 2016). Despite a relatively weak

affiliation with human environments, these mosquitoes can be vectors of malaria parasites. *An. daciae* may also be infected with agents of filariasis (Kronefeld et al. 2014). Since one of the main techniques of insect control is insecticide treatment, resistance of *An. messeae* s.l. malaria mosquitoes to different kinds of insecticides is interesting in practical terms, yet has not received the attention it deserves.

Malathion, an organophosphate insecticide, is widely used to control pests of forestry, agriculture, and gardening, as well as mosquitoes. Consequently, our study aimed at finding possible differences between the closely related *An. messeae* and *An. daciae* species with regard to malathion tolerance. Since the G119S mutation in the *ace-1* gene is important for organophosphate resistance development, we searched for this mutation in some populations of *An. messeae s.l.* and *An. beklemishevi*.

MATERIALS AND METHODS

Malathion resistance test

An insecticide resistance test was based on the standard technique of the World Health Organization (WHO 1981, 2005). We made six collections of *Anopheles maculipennis* complex larvae from two water ponds in the Novosibirsk suburb during the summer of 2016 (Figure 1). Second and 3rd instar larvae were cultivated for a few days until 3rd or early 4th instar. Groups of 10-60 individuals were selected and divided into different malathion concentrations, spending 24 h without food. We examined 24 such groups of individuals. At the end of the experiment, remaining specimens were fixed individually in ethanol. Larvae that died during the experiment were individually sampled and fixed. Air temperature in the room during experiments was 25-30° C. At first, we tested the malathion concentrations that were proposed by WHO (WHO 1981): 3.125, 0.625, 0.125 and 0.025 mg/L. As a control, we added ethanol to water (4 mL/L; this concentration was added to other glasses as the insecticide solvent). Larvae quickly died in 3.125 mg/L malathion. Therefore, we excluded this concentration from further experiments and added a 0.0625 mg/L group because it was similar to the concentration that killed 50% of individuals (LC_{50}).

Species affiliation

We used a standard ITS2 (second transcribed spacer of rRNA genes) polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (Novikov et al. 2004, Vaulin et al. 2018) to assign individuals to a species. DNA was isolated from individual larvae. We performed PCR of ITS2 by using primers based on Beebe and Saul (1995). Sequences were: 5'-TGTGAACTGCAGGACACAT-3' (forward) and 5'-TATGCTTAAATTCAGGGGGT-3' (reverse). The reaction mixture contained: 1 x PCR buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8 at 25° C) and 0.1% Tween-20), 4 mM $MgCl_2$, 0.4 mM dNTPs, 1 μM each primer, and one unit of Taq-polymerase. The thermal cycling profile included denaturation at 94° C for 1 min, annealing at 50° C for 1 min and polymerization at 72° C for 1 min (5 min for the last cycle); the number of cycles was 35.

We performed restriction enzyme digestion by combining 2 μL of PCR product and one unit of *BsrF5* I or *Sfr303* I restriction enzyme. The reaction was performed for at least 3 h. Restriction products were separated by electrophoresis with 2% agarose gels stained with ethidium bromide. Specimens with unclear patterns were studied repeatedly with an increased amount of enzyme or reaction time or alternative restriction enzyme. Overall, we studied 851 specimens from two water bodies and six collections. We failed to reveal species relationships for 59 individuals.

Statistics

We analyzed potential differences between species with regard to insecticide resistance using Pearson's chi-squared test performed for 0.125, 0.0625, and 0.025 mg/L malathion concentrations. LC_{50} was determined by probit analysis using the STATISTICA software package.

Table 1. Collections of malaria mosquito populations that were tested for the diagnostic mononucleotide substitution in the *ace-1* gene. The number of sequenced specimens for each group is shown in brackets.

Places and years of collections	Designation on Figure 1	Number of studied specimens		
		An. daciae	*An. messeae*	*An. beklemishevi*
Miass 2010	1	17 (1)	9 (1)	-
Miass 2014		1 (1)	-	-
Miass 2015		18 (1)	10 (1)	-
Kazakhstan, Kokshetau 2012	2	15 (1)	12 (1)	-
Novosibirsk 2016 (resistance test)	3	81 (2)	28 (2)	-
Chainsk 2010	4	21 (1)	6 (1)	5 (1)
Kazakhstan, Zyryanovsk 2012	5	-	27 (2)	-
Asino 2010	6	37 (1)	3 (1)	-
Teguldet 2015	7	4 (1)	7	3 (2)
Lensk 2011	8	-	43 (2)	-

Figure 1. Collection locations used for analysis of the diagnostic mononucleotide substitution in the *ace-1* gene. Locality designations are given in Table 1.

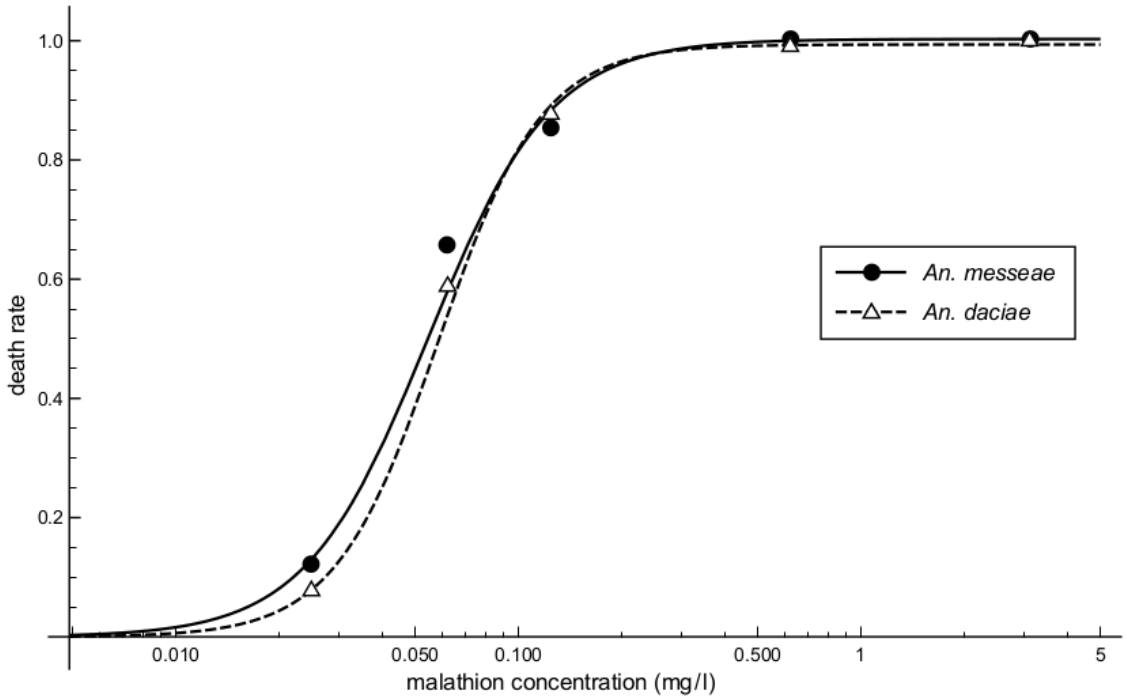

Figure 2. Relationship between malaria mosquito larvae mortality and malathion concentration. The mortality was corrected by the death rate in the control group.

Table 2. Accession numbers for sequences obtained from the EMBL DNA database. These sequences were used for comparison with the specimens of the *Anopheles maculipennis* group.

Species	Accession numbers
Anopheles stephensi	HG380320-HG380324, KP233478.
Anopheles sinensis	KF709027-KF709034, KF718282-KF718285, KY014600
Anopheles albimanus	AJ566402, AJ566403
Anopheles gambiae	KF771240-KF771247, KM875634-KM875637, KP165332-KP165384
Anopheles pseudopunctipennis	KY352490
Anopheles vestitipennis	KY412553
Anopheles vagus	KF718286

***ace-1* gene variability**

We sequenced the diagnostic fragment of the *ace-1* gene for 24 specimens from our *Anopheles* DNA collection. These specimens consisted of one *An. atroparvus* (a single specimen from a culture) and 23 specimens from nature (Table 1). We used the primers designed by Chang et al. (2014) for *An. sinensis*: 5'-GTGCG-ACCAT-GTGGA-ACC-3' (forward) and 5'- ACCAC-GATCA-CGTTC-TCCTC-3' (reverse). Sequencing was performed using resources from the Center for Collective Use Genomics (Siberian Branch, Russian Academy of Sciences; http://www.niboch.nsc.ru/doku.php/sequest). The sequences were submitted to the EMBL DNA database (numbers LT993740-LT993763). We compared the *ace-1* fragment, which directly adjoins the diagnostic site, for the studied species and DNA sequences for *Anopheles* species in the EMBL DNA database. The accession numbers for these sequences are presented in Table 2. Sequence alignment was performed with the CLUSTALW program (http://www.genome.jp/tools-bin/clustalw), and amino acid composition was studied using MEGA6 software (Tamura et al. 2013).

We performed a standard restriction analysis to search for diagnostic nucleotide substitutions (Chang et al. 2014) for 347 specimens (including sequenced *An. messeae* s.l. and *An. beklemishevi* specimens). PCR was performed with *ace-1* gene fragment primers (see above). One μL of PCR product was processed by the *Alu* I restriction enzyme. The *An. messeae* ITS2 PCR product was used as a control because it contains sites that are recognized by *Alu* I. We studied both living and dead specimens exposed to 0.0625 mg/L malathion, as well as specimens from other localities (Table 1, Figure 1). Specimens from the localities were studied for species relationships by PCR-RFLP ITS2 as described above.

The data on species composition from Miass (2010), Chainsk (2010) and Asino (2010) collections were published previously (Vaulin and Novikov 2012). Specimens that were used for the insecticide resistance test combined a portion of material from Vaulin et al. (2018) with the composition of malaria mosquito populations.

RESULTS

We examined the species composition of larval collections determined by ITS2 restriction analyses during the summer of 2016 in the suburb of Novosibirsk. An overwhelming majority of specimens, for which species was determined, were *An. messeae* s.l. Specifically, specimens included 607 *An. daciae*, 170 *An. messeae*, two *An. beklemishevi,* and 13 that were intermediate between *An. messeae* and *An. daciae* restriction spectra and were referred to as interspecific hybrids of *An. messeae* s.l. Thus, *An. daciae* was dominant in the localities during the study period. However, *An. messeae* s.l. species undergo known fluctuations in their proportions (Novikov 1997, Novikov and Vaulin 2014). For example, the proportion of those conspecific to *An. messeae* s.s. increased during a cold summer (Vaulin et al. 2018), but this species proportion decreased in Siberia due to global warming.

Data regarding mosquito survival after exposure to different malathion concentrations appear in Table 3 and

Table 3. Survival of different malaria mosquito species larvae in different malathion concentrations.

Malathion concentration mg/l	Number of survived / number of dead				
	An. daciae	*An. messeae*	Hybrids within *An. messeae* s.l.	*An. beklemishevi*	Species relation was not defined
3.125	0/41	0/11	0/1	-	0/1
0.625	1/107	0/29	0/1	-	0/20
0.125	15/107	5/29	0/2	-	1/11
0.0625	42/60	11/21	2/2	-	5/8
0.025	107/9	29/4	3/1	-	7/0
Negative control	116/2	30/1	1/0	2/0	6/0

Table 4. Distribution of malathion resistance in different mosquito species larvae. If several specimen groups were studied, then their LC_{50} values are separated by commas. For the *Cx. quinquefasciatus* study (Selvi et al. 2005), minimal and maximal values from ten groups under selection are provided.

Species	LC_{50} value mg/L		Reference
	Without selection	Culture under selection	
Culex quinquefasciatus	0.038, 0.292	-	Curtis, Pasteur, 1981
	0.08, 3.3, 7.5	84	Bisset et al., 1990
	0.016	0.860	Hamdan et al., 2005
	0.012	0.935-1.579	Selvi et al., 2005
Aedes aegypti	0.23, 0.47, 0.65	-	Mazzarri, Georghiou 1995
	0.060	0.298	Hamdan et al., 2005
	0.060	0.377	Hidayati et al., 2011
Aedes albopictus	0.124	1.139	Hamdan et al., 2005
Anopheles stephensi	0.004, 0.01	0.866, 0.938	Chitra, Pillai, 1984.
Anopheles stephensi	0.18	1.6	Scott, Georghiou, 1986
Anopheles messeae	0.048	-	This study
Anopheles daciae	0.052	-	This study

Figure 2. At 3.125 mg/L, all specimens died. A single *An. daciae* specimen survived at 0.625 mg/L, while more than half the individuals died if the malathion concentration was 0.0625 mg/L. There was no difference between *An. messeae* and *An. daciae* with regards to survival at 0.125, 0.0625, or 0.025 mg/L malathion. The LC_{50} value for the specimen mixture was 0.052 mg/L. Further, the LC_{50} value was not significantly different for *An. messeae* (0.055 mg/L) and *An. daciae* (0.048 mg/L). *An. beklemishevi* specimens were too rare in the Novosibirsk suburb and thus we could not estimate its malathion resistance.

As mentioned previously, the G119S mutation in *ace-1* is associated with organophosphate and carbamate resistance (Weill et al. 2004). We studied *ace-1* sequences for 24 specimens related to four Palearctic species of the *Anopheles maculipennis* group (*An. atroparvus*, *An. beklemishevi*, *An. messeae,* and *An. daciae*) to determine a possible relationship between the level of malathion resistance and gene structure. Analyses revealed that the individuals were different from many other mosquito species at the diagnostic site (Figure 3). The triplet GGC codes for glycine at the 119th position of ACE-1 in mosquitoes from *Anopheles gambiae*, *An. sinensis*, *Anopheles albimanus,* and *Culex pipiens* natural populations (Weill et al. 2004, Chang et al. 2014). This triplet is replaced by the AGC triplet in resistant mutants via a single mononucleotide substitution. The Palearctic *An. maculipennis* complex species harbored

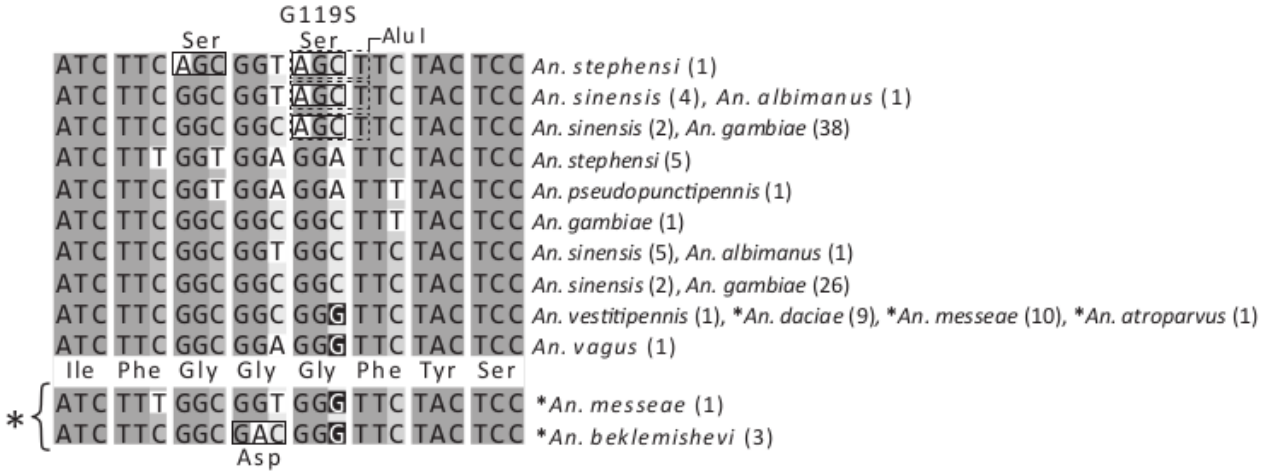

Figure 3. Nucleotide and amino acid sequences of the *ace-1* gene diagnostic fragment for four Palearctic species of the *Anopheles maculipennis* group and seven other *Anopheles* species. The frames designate triplets that are related to changes in the consensus amino acid sequences. The dotted line designates the *Alu I* recognition site. The gray color shades designated nucleotide variations. Numbers of specimens in each group are shown in brackets. Asterisks represent the *Anopheles maculipennis* group species examined in the study and variants of nucleotide sequences which were revealed during the study.

a GGG glycine triplet at the position. With this triplet, at least two mononucleotide substitutions would be required to change it to either AGC or AGT that codes for serine. Thus, a resistance mutation in *ace-1* would be difficult to develop in the *Anopheles maculipennis* mosquito group. It is interesting that the GGG triplet also appears in *An. vestitipennis* and *An. vagus*. These two species are part of different *Anopheles* subgenera (Harbach 2004). It seems that this triplet appeared and became fixed in different branches of *Anopheles* several times. In *An. sinensis*, the variation pattern of the diagnostic triplet, and the triplet situated just before it, indicates either two independent origins for the G119S mutation or a parallel origin of identical substitutions in the previous triplet (Figure 3).

We analyzed our mosquito collection with PCR-RFLP to determine the potential presence of the *An. messeae* s.l. G119S mutation. Collections originated from six localities in the Asian part of Russia and two from Kazakhstan (Figure 1). For Miass in Chelyabinsk oblast, we studied collections over a few years. This geographic (and to a lesser extent) time distribution allowed us to study specimens with sufficient variable genomes. This design increased the possibility of detecting the diagnostic substitution compared to analyses of collections from Novosibirsk in 2016 alone. Identifying this kind of mutation in any locality has important epidemical implications. Nevertheless, we were unable to find the G119S mutation even by restriction analysis of 347 specimens.

DISCUSSION

Large-scale insecticide usage began in the late 1940s in Europe in order to control mosquito vectors. Later, insecticides, mostly DDT, were extensively used to control malaria and agricultural pests. The use of newer insecticides, and consequent resistance development to earlier insecticides, led to the necessity to determine effective insecticide dosages (Wood 1965, Birley et al. 1987, Georghiou et al. 1987). These studies reported estimations of insecticide resistance in different mosquito groups. Notably, measurements of larval and adult resistance use different techniques (WHO 2005) their resistance can occur by different mechanisms. For example, it was shown for *An. stephensi* that larval resistance to organophosphates may not lead to resistant adults (Chitra and Pillai 1984). However, analyses of molecular resistance mechanisms were not developed at the time that paper was published.

From the characteristics of larval mosquito resistance to malathion shown in Table 4, it appears that in the laboratory insecticide resistance can be altered from to one to two orders of magnitude compared to natural materials and stocks that were cultured without selection.

Even in the absence of laboratory selection, there can be significant variation in LC_{50} values among different members of a single species (Table 4). This kind of variation was found for *Cx. quinquefasciatus* (0.012 - 7.5 mg/L) and *Aedes aegypti* (0.060 - 0.65 mg/L) (Bisset et al. 1990, Mazzarri and Georghiou 1995, Selvi et al. 2005, Hidayati et al. 2011). Interspecies resistance variability may be due to test conditions, but only to a small degree. The most important factor for insecticide resistance variability is likely to be differential natural habitat pollution by xenobiotics. Moreover, as shown in Table 4, the tests were conducted at different times and for different populations. As a result, xenobiotic resistance mechanisms could spread to different populations. For example, the *Ae. aegypti* stock, which was long cultivated under laboratory conditions, was two to three times less resistant to malathion than stocks obtained from natural populations, where they could interact with pesticides (Mazzarri and Georghiou 1995).

Our overall LC_{50} estimation for *An. messeae* s.l. (0.052 mg/L) is in the range of variability for *An. stephensi* and *Cx. quinquefasciatus* without laboratory selection. The LC_{50} values for *An. messeae* and *An. daciae* are similar to the minimal LC_{50} value for *Ae. aegypti* (0.060 mg/L), and it is more than two times less than the value for *Aedes albopictus* (0.124 mg/L). Notably, we did not find any differences between LC_{50} values for *An. messeae* and *An. daciae*, but other species exhibited highly significant interspecific malathion resistance variation. Malathion resistance in mosquito groups subjected to laboratory selection was much higher (LC_{50} 0.298 - 84 mg/L) than the resistance of mosquitoes in our study. Thus, our *An. messeae* s.l. collections were not characterized by high malathion resistance and this insecticide could be used successfully for *An. messeae* s.l. control.

Insecticide resistance may be related to genetic and corresponding biochemical variations. Modification of the main insecticide target appears to be a main mechanism of resistance (ffrench-Constant 2013). In particular, a mononucleotide substitution in the *ace-1* gene, responsible for malathion resistance, belongs to this type of mechanism (Weill et al. 2004). The modified gene product has somewhat reduced activity, but it exhibits markedly reduced interaction with insecticides. Thus, in the absence of insecticide, these kinds of mutations became a genetic load component. The *ace-1* mutation reduces the corresponding enzyme activity approximately 60% in *Cx. pipiens*. Sufficient selection against this mutation was shown for *Cx. pipiens* and *An. albimanus* (Weill et al. 2004). The results from our study on the structure of the diagnostic *ace-1* gene fragment in the *An. messeae* s.l. collections revealed that this resistance mechanism is hindered by features of the nucleotide sequence at the mutation site (Figure 3).

Insecticide resistance may also develop by genetic mutations that increase activity of enzymes that degrade xenobiotics (WHO 2013). For example, carboxylase activity is the main factor of insecticide resistance in *An. sinensis* populations (Chang et al. 2014). Nevertheless, abundant evidence about the important role of the G119S *ace-1* gene mutation in organophosphate resistance leads us to consider that this mononucleotide substitution is the main mechanism of malathion resistance.

Anopheles messeae s.l. was not previously examined for resistance to malathion and other broadly used insecticides. However, extensive studies on resistance to the *Bacillus thuringiensis israelensis* (Bti) toxin were performed for this mosquito taxon (Gordeev and Burlak 1991) before dividing

the group into *An. messeae* and *An. daciae*. They aimed to detect the influence of chromosomal inversions combinations on the degree of toxin resistance. For *An. messeae* s.l., there are three main X-chromosome variants (XL_0, XL_1, and XL_2), two second chromosome variants ($2R_0$ and $2R_1$) and two variants in each third chromosome arm ($3R_0$, $3R_1$, $3L_0$, and $3L_1$; Stegnii 1991). Furthermore, there are several local X-chromosome variants identified in the European part of Russia, one of which, XL_4, occurs regularly (Moskaev et al. 2016). *An. messeae* s.l. species differ from each other by dominant chromosomal variants (Novikov and Shevchenko 2001). There is a similar divergence pattern in the abundance of chromosomal rearrangements for the afrotropical mosquito species of *An. gambiae* and *An. coluzzii* (Lanzaro and Lee 2013). Thus, it is possible to interpret results obtained in terms of chromosomal rearrangements to results in species relationships. It was demonstrated that individuals with inversion variants XL_0, $2R_0$, $3R_0$, and $3L_0$ are more resistant to Bti toxin than specimens with variants XL_2, $2R_1$, $3R_1$, and $3L_1$ (Gordeev and Burlak 1991). Notably, the former group of rearrangements is characteristic for *An. daciae* and the latter is characteristic of *An. messeae* (Vaulin et al. 2018). Based on these differences, *An. daciae* seems to be more resistant to the Bti toxin than *An. messeae*.

Conspecifics to *An. daciae* appear to be more associated with human environments and more often feed on human blood compared to *An. messeae* (Danabalan et al. 2013, Novikov and Vaulin 2014). When we planned the experiment, we suspected that the relatively anthropophilic *An. daciae* species would be more resistant to malathion than *An. messeae* because it would be more often exposed to insecticides. Similarly, *Drosophila melanogaster*, which is highly associated with human environments but is not a main insecticide target, possesses resistance mechanism to many insecticides, and this mechanism is broadly spread within populations (Daborn et al. 2002). There is evidence for a similar associated selection for *An. albimanus* related to cotton plantation pest control (Ariaratnam and Georghiou 1971). Insecticides are used in the studied area (Novosibirsk suburb) partially to protect against annoying gnats (Mirzaeva 2008). It is reasonable to expect that most anthropophilic mosquito species will exhibit greater selection for insecticide resistance. However, our results did not demonstrate this relationship for the specimens we studied.

We cannot exclude a divergence within *An. messeae* s.l. with regard to genes that are associated with xenobiotic inactivation. Since a large portion of genomes of this species pair includes polymorphic chromosomal inversions, it is possible that different alleles of genes related to resistance are associated with different chromosomal rearrangements. Future studies on *An. messeae* s.l. resistance to different insecticides, and chromosomal inversion combinations and variability in genes that are potentially associated with insecticide resistance, will allow for a better understanding of the mechanisms that different organisms use for protection from insecticides.

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