

The distribution of strains of endosymbiotic bacteria *Wolbachia pipiensis* in natural populations of *Culex pipiens* mosquitoes (Diptera: Culicidae)

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Abstract

Wolbachia is a group of maternally inherited endosymbiotic bacteria that infect and induce cytoplasmic incompatibility in a wide range of arthropods. *Wolbachia* species are involved and play a significant role in some evolutionary processes, e.g. those of rapid speciation. The mosquito *Culex pipiens* displays the greatest variability of cytoplasmic incompatibility crossing types compared with other arthropods suggesting differential infection by multiple *Wolbachia pipiensis* strains. We investigated the distribution of these strains based on the polymorphism of transposable element *Trl* in natural populations of the *C. pipiens*, as well as *C. torrentium*, from Russian Federation, Republic of Kazakhstan, Kyrgyz Republic and Republic of Belarus. No *C. torrentium* were found infected. Infection rates of different strains of *Wolbachia* in populations of typical and *molestus* forms of *C. pipiens* are compared and discussed.

Keywords: *Culex pipiens*, cytoplasmic incompatibility, transposable element, *Wolbachia*

Introduction

Bloodsucking mosquitoes are constituent elements of a number of various parasitic systems mediating specific as well as purely mechanical transmission of protozoan, bacterial and virus diseases, both human and animal. Prior to the decision on the epidemic importance of a species precise identification of the vector is necessary, first of all it is true for the species of the *Culex pipiens* complex. The *Culex pipiens* complex includes some species of controversial taxonomic status which possess only minor morphological but great biological differences, including the ability to transmit human and animal diseases. In this respect the species *C. pipiens* Linnaeus is of particular concern because its two forms, the typical and *molestus* forms, have different biological characteristics. *Culex torrentium* Martini a related sympatric species, is also of interest. The major morphological characteristics which allow identification of the typical and

molestus forms are the siphon index and the number of dorsomental teeth of larvae (Vinogradova, 1997). Only males are used for the identification of *C. torrentium*, which can be reliably distinguished from *C. pipiens* using details of the male genitalia (Kruppa, 1988; Vinogradova, 1997).

We investigated the distribution of endosymbiotic bacteria *Wolbachia* Hertig strains in natural populations of the typical and *molestus* forms of *C. pipiens*, and *C. torrentium*. Due to wide spread occurrence of the bacteria and reproductive effects which they induce, particularly cytoplasmic incompatibility, *Wolbachia* species are involved and play a significant role in some evolutionary processes, e.g. those of rapid speciation (Hoy, 1994; Werren, 1997; Coyne & Orr, 1998). The mosquito *C. pipiens* displays the greatest variability of cytoplasmic incompatibility crossing types compared with other arthropods (Laven, 1967; Barr, 1980; Magnin *et al.*, 1987). The major

hypotheses for the highly complex cytoplasmic incompatibility patterns in *C. pipiens* are the presence of different *Wolbachia* strains or the occurrence of uninfected insects in natural populations (Duron *et al.*, 2005). However, no polymorphism was observed of the *ftsZ* gene sequences (Guillemaud *et al.*, 1997) and 16S rRNA of *W. pipipientis* (Rousset *et al.*, 1991). Duron *et al.* (2005) identified five *W. pipipientis* strains by analyzing the polymorphism of *Trl*, a transposable element of the IS5 family.

Material and methods

Specimens of *C. torrentium* and the typical and *molestus* forms of *C. pipiens* from both open and isolated, both natural and artificial sites of the Russian Federation (Tomsk, Novosibirsk, Altay and Krasnodar Regions), the Republic of Kazakhstan and the Kyrgyz Republic were collected as larvae or pupae during the period 2000-2007. Additionally, in 2005-2007 collection and recording of bloodsucking mosquitoes of *C. pipiens* were performed in three forest subzones in the territory of five administrative districts, namely Minsk, Vitebsk, Grodno, Mogilevsk, and Gomelsk regions of the Republic of Belarus.

Molecular markers used to identify members of the *C. pipiens* complex were as follows: SCAR-markers (Khrabrova *et al.*, 2006), second intron of acetylcholinesterase 2 gene (*ACE2*) (Smith & Fonseca, 2004), and RFLP-PCR of cytochrome oxidase I gene of mitochondrial DNA (3'- and 5'-ends, *COI* 3' и *COI* 5') (Vinogradova & Shaikevich, 2005; Shaikevich, 2007]. Primers 81F и 691R (Zhou *et al.*, 1998) were utilized to define infection by *Wolbachia*. Identification of *W. pipipientis* variants was carried out based on *Trl* sequence according to Duron *et al.* (2005).

Results and Discussion

A total of 2,565 larvae and pupae of *C. torrentium* (853 specimens), typical *C. pipiens* (985 specimens) and *C. p. form molestus* (727 specimens) from 75 populations were collected

and investigated. Both homogeneous and mixed populations were revealed. *Culex torrentium* is common in the Tomsk region (more than half of the total number of *C. torrentium* samples, most of which homogeneous). *Culex torrentium* has also been found in the Novosibirsk and Altay regions, the Republic of Kazakhstan, Kyrgyz Republic and the Republic of Belarus. *Culex p. form molestus* was abundant in all places studied. Mixed collections were grouped into four types: a) *C. torrentium* and typical *C. pipiens* (the Tomsk, Novosibirsk and Altay Regions, Republic of Kazakhstan, Republic of Belarus); b) *C. torrentium* and *C. p. form molestus* (the Tomsk region); c) typical *C. pipiens* and *C. p. form molestus* (the Novosibirsk, and Krasnodar regions, Republic of Kazakhstan, Republic of Belarus); d) *C. torrentium*, typical *C. pipiens* and *C. p. form molestus* (the Tomsk region, Kyrgyz Republic, Republic of Belarus).

No *C. torrentium* were found infected by *Wolbachia*, but $98.2 \pm 0.5\%$ of the populations of *C. p. form molestus* (notably, uninfected *C. p. form molestus* individuals are present only in Belarus populations) and 34.5–100 % individuals from populations of typical *C. pipiens* were infected (on the average $88.0 \pm 1.0\%$). It should be noted that most of typical *pipiens* mosquitoes were collected in the territory of Belarus, thus the analysis of these populations was performed apart from other populations. In Belarus populations $81.4 \pm 1.6\%$ of typical *pipiens* mosquitoes were infected. Infection rates for some typical *pipiens* populations are summarised in Table 1. The data are in accordance with those obtained by Vinogradova *et al.* (2007) for the European part of the Russian Federation: no infections in *C. torrentium* and 70 – 90 % in the typical and *molestus* forms of *C. pipiens* mosquitoes were infected (Vinogradova *et al.*, 2007). However, according to Duron *et al.* (2005) 100% of *C. p. molestus* and *C. p. pipiens* in Portugal, Spain, France, Italy, Switzerland, Belgium, Great Britain, Netherlands, Greece, Turkey, Cyprus, Tunisia, and Australia are infected by *W. pipipientis*.

Table 1. The distribution of *W. pipipientis* strains in populations of typical *C. pipiens*.

Abbreviations used: RF – Russian Federation, RK – Republic of Kazakhstan, KyrR – Kyrgyz Republic, BR – Republic of Belarus, TR – Tomsk region, NR – Novosibirsk region, AR – Altay region, KR – Krasnodar region; f – frequency, s_f – sample rate error, n – sample number

Locality	Co-ordinates		Collection date, D/M/Y	n	Number of <i>C. p. pipiens</i> individuals infected by <i>W. pipipientis</i> , f $\pm s_f$ (%)	Number of <i>C. p. pipiens</i> individuals infected by wpip2-A, f $\pm s_f$ (%)	Number of <i>C. p. pipiens</i> individuals infected by wpip3, f $\pm s_f$ (%)
	N	E					
RF, TR, Moryakovsky Zaton	56°	84°	03.08.2003	24	100	0	100
RF, NR, Novosibirsk	55°	83°	27.08.2006	9	77.8 \pm 13.8	0	100
RF, NR, Troitskoe	54°	78°	28.09.2007	47	100	14.9 \pm 5.2	85.1 \pm 5.2
RF, AR, Len'ki I	53°	80°	22.08.2000	35	100	40.0 \pm 8.2	60.0 \pm 8.2
RF, AR, Len'ki II	53°	80°	18.08.2001	16	62.5 \pm 12.1	0	100
RF, KR, Sochi	43°	39°	14.07.2006	46	100	47.8 \pm 7.3	52.2 \pm 7.3
RK, Semipalatinsk I	50°	80°	16.08.2004	72	100	0	100
RK, Semipalatinsk II	50°	80°	23.08.2006	29	100	0	100
KyrR, Mailuu-suu I	41°	72°	15.06.2006	42	100	0	100
KyrR, Mailuu-suu II	41°	72°	15.06.2006	48	100	0	100
BR, Minsk I	54°	27°	25.09.2006	28	46.4 \pm 9.4	92.3 \pm 7.4	7.7 \pm 7.4
BR, Minsk II	54°	27°	15.11.2006	47	65.9 \pm 6.9	90.3 \pm 5.3	9.7 \pm 5.3
BR, Minsk III	54°	27°	15.11.2006	43	69.8 \pm 7.0	83.3 \pm 6.8	16.7 \pm 6.8
BR, Stolbcy I	53°	27°	19.11.2006	40	95.0 \pm 3.4	52.6 \pm 8.1	47.4 \pm 8.1
BR, Minsk IV	54°	27°	29.09.2006	46	91.3 \pm 4.1	90.5 \pm 4.5	9.5 \pm 4.5
BR, Stolbcy II	53°	27°	19.11.2006	47	91.5 \pm 4.0	58.1 \pm 7.5	4.9 \pm 7.5
BR, Borisov	54°	28°	19.11.2006	48	97.9 \pm 2.1	93.6 \pm 3.6	6.4 \pm 3.6
BR, Braslav I	55°	27°	21.11.2006	11	81.8 \pm 11.6	66.7 \pm 15.7	33.3 \pm 15.7
BR, Slonym I	53°	25°	15.08.2005	17	100	0	100
BR, Minsk V	54°	27°	05.12.2006	45	88.9 \pm 4.7	77.5 \pm 6.6	22.5 \pm 6.6
BR, Braslav II	55°	27°	05.12.2006	22	100	86.4 \pm 7.3	13.6 \pm 7.3
BR, Stolbcy III	53°	27°	29.08.2005	8	100	0	100
BR, Minsk VI	54°	27°	11.09.2005	15	100	0	100
BR, Minsk VII	54°	27°	07.12.2006	21	52.4 \pm 10.9	90.9 \pm 8.7	9.1 \pm 8.7
BR, Grodno	54°	24°	21.06.2007	14	57.1 \pm 13.2	62.5 \pm 17.1	37.5 \pm 17.1
BR, Ozery	54°	24°	13.07.2007	9	100	0	100
BR, Boyary	54°	25°	11.07.2007	22	100	0	100
BR, Slonym II	53°	25°	15.07.2007	29	34.5 \pm 8.8	70.0 \pm 14.5	30.0 \pm 14.5
BR, Shchuchin	53°	25°	28.06.2007	30	100	0	100
BR, Brosty	53°	24°	24.07.2007	13	46.2 \pm 13.8	100	0

Transposable element *Tr1* polymorphism discovered by Duron *et al.* (2005) may be presence or absent and exhibit sequence variation when present. Five *W. pipipientis* strains have been described based on presence/absence and sequence variation: wpip3, which lacks *Tr1*; wpip1, wpip2-A and wpip4, which contain distinct *Tr1* alleles; wpip2-B, which contains the same *Tr1* sequence as wpip2-A, but at a distinct locus (Duron *et al.*, 2005).

Two *Wolbachia* strains, wpip3 and wpip2-A, occur in the populations studied. Populations of the *molestus* form carry the wpip3 strain only (all infected mosquitoes), whereas typical *pipiens* carry both wpip3 and wpip2-A. The wpip2-A strain has been found in the Novosibirsk, Krasnodar and Altay Regions, and Republic of Belarus. The Tomsk Region, Kazakhstan and Kyrgyz populations are infected by the wpip3

strain only but this result may be due to low number of populations of typical *pipiens* studied in these regions (Table 1). The wpip3 strain is the most abundant in typical *pipiens* populations: $60.8 \pm 1.7\%$ of all infected mosquitoes, but in different populations this value varies greatly: from 6.4 to 100 %; wpip2-A accounts for $39.2 \pm 1.7\%$. The wpip2-A strain is more common in Belarus *C. p. pipiens* populations ($61.5 \pm 2.3\%$) (Table 1).

Previously the wpip3 strain was found in Central and South America, Europe, Africa, Asia and Oceania, but not in North America. The wpip3 strain is the most common *Wolbachia* strain found in populations of typical and *molestus* forms of *C. pipiens* and the closely related *C. quinquefasciatus*. The wpip1 strain is prevalent in North America and overlaps with wpip3 in Spain, Portugal, and southern France. The wpip2-A strain is present in Great Britain populations only, and wpip4 only in Cyprus. It is noteworthy that in Great Britain *C. p.* form *molestus* was infected by wpip3 and typical *pipiens* by wpip2-A (Duron *et al.*, 2005). Our data indicate that populations of the *molestus* form are infected by wpip3, whereas those of the typical form are infected by two *Wolbachia* strains, wpip2-A and wpip3, which occur sympatrically. Two types of cytoplasmic incompatibility are known to exist: unidirectional (infected male and non-infected female) and bidirectional (male and female harbor different strains of *Wolbachia* that are mutually incompatible) (Werren, 1997). It is predicted that the coexistence of multiple bacterial variants is not stable within a population if these variants generate cytoplasmic incompatibility (Rousset *et al.*, 1992). This would imply that either wpip2-A and wpip3 do not generate cytoplasmic incompatibility in these areas, or that the coexistence of wpip2-A and wpip3 is transient. It is not clear whether the *molestus* and typical forms of *C. pipiens* readily mate in natural conditions.

We have shown that the *molestus* and typical forms of *C. pipiens* are not biotopically isolated, on the contrary, their co-habitation is usual, particularly in spring and winter. On the other hand, the absence of wpip2-A in populations of form *molestus* may suggest the existence of isolation mechanisms. However, it should be remembered that identification of specimens of the *molestus* and typical forms of *C. pipiens* is based on the mitochondrial marker, and thus does not allow separation of hybrid individuals. Therefore, the problem of natural hybridization between the *molestus* and typical forms remains

unresolved, and requires further exploration. The main obstacle here is the lack of genomic markers for the unequivocal identification of the *molestus* and typical forms of the species.

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