

LOW PREVALENCE OF *WOLBACHIA* INFECTION IN UKRAINIAN POPULATIONS OF *DROSOPHILA*

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Aim. The aim of this study was to determine the *Wolbachia* infection prevalence among *Drosophila* species that are common in Ukraine. **Methods.** The total of 203 imagoes, representatives of seven *Drosophila* species collected from seven localities in Ukraine were screened for *Wolbachia* via PCR assay. **Results.** We found *Wolbachia* infection only in one individual of *Drosophila testacea* that was collected in the Chernobyl Exclusion Zone. **Conclusions.** In Ukraine, the examined *Drosophila* species are characterised by a low prevalence of *Wolbachia* infection. This research, together with previously reported infections in *D. melanogaster* and *D. simulans* populations, indicate that *Wolbachia* infects 3 out of 9 *Drosophila* species surveyed in Ukraine.

Keywords: *Wolbachia*, *Drosophila*, natural populations, *D. testacea*, endosymbiont.

Wolbachia are obligate intracellular endosymbionts of arthropods that is widespread in nature [1]. Given the ability of *Wolbachia* to manipulate host reproduction [1], recent studies highlight the importance to research relationships between endosymbionts and their arthropod hosts in nature [2, 3]. Nonetheless, most of such studies to date were either conducted on model organisms [4], or were related to pest control strategies [5], and thus even when examining multiple host species often lack biogeographical and ecological context [3, 6, 7]. *Wolbachia* are thought to infect from 20 to 50% of all the insect species, however these estimates are far from consensus, as actual prevalence of infection in nature remains to be unknown [8, 9]. Moreover, with just few exceptions [10], there is a lack of studies systematically examining *Wolbachia* infection status in multiple host species that co-occur and interact within a given habitat.

The presence of *Wolbachia* has been confirmed in multiple *Drosophila* host species around the world, for example, in *Drosophila melanogaster*; *D. simulans*, *D. suzukii* and *D. ananassea*, whereas it has not been detected in a wide range of other species, including *D. immigrans*, *D. repleta*, *D. obscura*, etc. [6, 7, 10, 11]. In Ukraine, *Wolbachia* are widely distributed in the natural populations of *D. melanogaster* [12] and *D. simulans* [13]. However, for other *Drosophila* species that are common in Ukraine [14], *Wolbachia* infection status remains to be unknown. Therefore, here we investigate the presence of *Wolbachia* infection in different *Drosophila* species that are common in Ukraine.

Materials and Methods. Flies were sampled from seven localities in Ukraine (Yalta, Odesa, Uman, Kharkiv, Pyriatyn, Varva, Chornobyl) during August – October of 2015. A brief description of the sampled localities and their GPS coordinates can be found in Serga et al. [13]. Captured flies were assigned to the respective taxonomical group based on the external morphological features [15].

DNA extraction was performed from whole-bodies of adult flies of each species (*D. repleta*, *D. hydei*, *D. obscura*, *D. subobscura*, *D. testacea*, *D. busckii* and *D. immigrans*) using the high-salt method [16]. *Wolbachia* infection was tested by PCR using a set of primers to bacterial *16S rRNA* gene (5'-CATACCTATTCGAAGGGATAG, 5'-AGCTTCGAGTGAACCCAATTC) [17] and *wsp* gene (81F 5'-TGGTCCAATAAGTGATGAAGAAAC, 691R 5'-AAAAATTAAACGCTACTCCA) [18]. To confirm obtained results, each PCR was repeated twice.

Table 1

***Wolbachia* infection among different *Drosophila* species collected from natural populations in Ukraine**

Population	Yalta		Odesa		Uman		Kharkiv		Varva		Pyriatyn		Chornobyl	
	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>
<i>D. repleta</i>	32	10	0	0	0	0	0	0	42	10	5	5	136	30
<i>D. hydei</i>	0	0	10	10	41	10	0	0	1	1	0	0	99	50
<i>D. obscura</i>	0	0	0	0	1	1	2	2	0	0	0	0	21	10
<i>D. subobscura</i>	0	0	0	0	0	0	0	0	0	0	0	0	2	2
<i>D. testacea</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	1*
<i>D. busckii</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>D. immigrans</i>	7	7	37	20	1	1	8	8	0	0	5	5	19	19

N – the total number of collected individuals; *n* – number of individuals that had been tested;

* *Wolbachia* positive sample

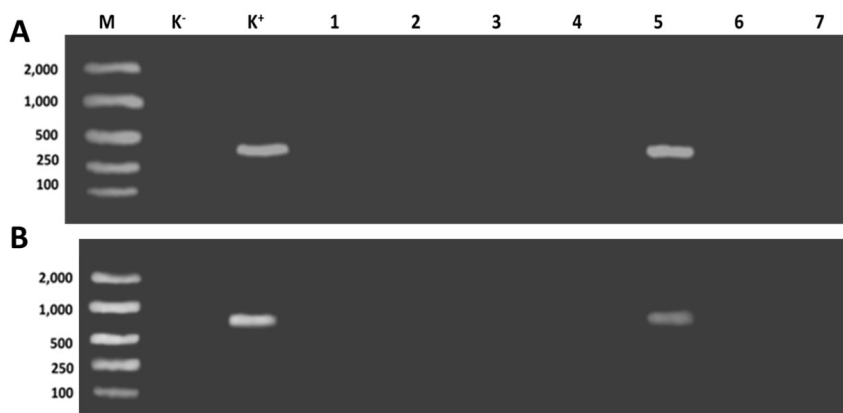


Fig. 1. Gel picture displaying PCR products of the 438 bp *16S rRNA* (A) and 632 bp *wsp* (B) gene fragments, used for detection of *Wolbachia* infection
Lanes: M – 100 bp DNA Ladder “New England BioLabs”; K⁻ – negative control;
K⁺ – positive control *D. melanogaster*; 1 – *D. subobscura*; 2 – *D. repleta*; 3 – *D. hydei*;
4 – *D. obscura*; 5 – *D. testacea*; 6 – *D. busckii*; 7 – *D. immigrans*.

Results. We have analyzed 203 imagoes, which belong to seven *Drosophila* species from seven localities in Ukraine to identify the presence of *Wolbachia* infection (Table 1). Examples of gel pictures displaying the PCR products of the *16S rRNA* and *wsp* gene fragments are presented in Figure 1. From all of the analyzed samples, only one DNA sample of *D. testacea* collected from the Chernobyl Exclusion Zone was positive for *Wolbachia*.

Discussion. Microorganisms have diverse implications for health, survival, fitness and adaptation of their animal hosts [1, 11]. *Wolbachia* are considered one of the most highly widespread bacteria among insects [8]. However, while *Wolbachia* are common among insects, its infection frequency and thus actual bacteria prevalence in nature are not ubiquitous and thus can be relatively low [3]. Indeed, the *Drosophila* species we surveyed in the present study are characterised by a low prevalence of *Wolbachia* infection. Only one individual of *D. testacea* was confirmed to be positive for *Wolbachia* infection out of more than 200 samples spanning across seven *Drosophila* species. Interestingly, *Wolbachia* has been previously reported in this species only once in a natural population located in France [19], despite the attempts to detect it in England [10] and Germany [20]. *Wolbachia* infects other species within *D. testacea* species group, such as *Drosophila neotestacea* [21] and *Drosophila orientacea* [20], although these species were not previously found from Ukraine.

The low prevalence of *Wolbachia* infection in other tested *Drosophila* species from Ukraine may be due to specific ecological conditions at the sampling localities, which hinder *Wolbachia* distribution in the surveyed populations, although at the same localities *Wolbachia* infection frequencies are consistently high for *D. melanogaster* (43–78%) [12] and *D. simulans* (100%) [13]. Alternatively, negative status of *Wolbachia* infection in these species indicate that *Wolbachia* are not generally typical for these species in Ukraine and elsewhere. Indeed, other studies have also found no evidence for *Wolbachia* infection in six out of seven species examined here [6, 7, 10, 20].

Recent studies suggest that distribution and the overall prevalence of *Wolbachia* in nature might be overestimated [3]. Our results further corroborate these findings, at least for the *Drosophila* species surveyed in Ukraine. Thus, we suggest that future research should include wide range of host species to test whether it is a more general pattern similar across other insects.

Conclusions. While in Ukraine *Wolbachia* infection is widespread among the natural populations of *D. melanogaster* [12] and *D. simulans* [13], it was only detected in *D. testacea* and in no other *Drosophila* species examined in the present study. Therefore, *Wolbachia* infects only 3 out of 9 *Drosophila* species surveyed in Ukraine.

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НЕЗНАЧНЕ ПОШИРЕННЯ ІНФІКУВАННЯ *WOLBACHIA* В ПРИРОДНИХ ПОПУЛЯЦІЯХ *DROSOPHILA* В УКРАЇНІ

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Резюме

Мета. Метою роботи було оцінити рівень інфікування ендосимбіотичною бактерією *Wolbachia* представників природних популяцій різних видів *Drosophila*, які зустрічаються в Україні. **Методи.** На наявність *Wolbachia* було протестовано методом ПЛР 203 імаго 7 видів дрозофіл, які були зібрані в природі в 7 локалітетах України. **Результати.** *Wolbachia* була виявлена лише у однієї особини *Drosophila testacea*, яка була відловлена в Чорнобильській зоні відчуження. **Висновки.** Ендосимбіотична бактерія *Wolbachia* має незначне розповсюдження серед вивчених видів дрозофіл. Разом з раніше описаним інфікуванням в популяціях *Drosophila melanogaster* та *Drosophila simulans* вона ідентифікована в 3 з 9 досліджених видів дрозофіл в Україні.

Ключові слова: *Wolbachia*, *Drosophila*, природні популяції, *D. testacea*, ендосимбіонт.

НЕЗНАЧИТЕЛЬНОЕ РАСПРОСТРАНЕНИЕ ИНФИЦИРОВАНИЯ *WOLBACHIA* В ПРИРОДНЫХ ПОПУЛЯЦИЯХ *DROSOPHILA* В УКРАИНЕ

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Резюме

Цель. Целью работы было оценить уровень инфицирования ендосимбиотической бактерией *Wolbachia* представителей природных популяций различных видов *Drosophila*, которые встречаются в Украине. **Методы.** На наличие *Wolbachia* были протестированы методом ПЦР 203 имаго 7 видов дрозофил, собранные в природе в 7 локалитетах Украины. **Результаты.** *Wolbachia* идентифицирована только у одной особи *Drosophila testacea*, отловленной в Чернобыльской зоне отчуждения. **Выводы.** *Wolbachia* не является распространенной бактерией среди исследованных видов дрозофил. Вместе с ранее описанным инфицированием в популяциях *Drosophila melanogaster* и *Drosophila simulans* она идентифицирована в 3 из 9 изученных видов дрозофил в Украине.

Ключевые слова: *Wolbachia*, *Drosophila*, природные популяции, *D. testacea*, эндосимбионт.

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