υ Γ ϱ 575.17.+591.557:595.773.4

Short communication

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

S.V. Serga[1], P.A. Kovalenko[1], N.V. Gora[1], A.V. Lavrinienko[2], S.V. Demidov[1], F. Mestres[3], M. Pascual[3], I.A. Kozeretska[1]

[1]Taras Shevchenko National University of Kyiv,
64 Volodymyrska str, Kyiv, 01601, Ukraine
[2]Department of Ecology and Genetics, University of Oulu, 90014, Finland
[3]Universitat de Barcelona, Facultat de Biologia, Departament de Genètica, Barcelona, E-08028, Spain
e-mail: svitlana.serga@gmail.com

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 imago, 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 Chornobyl 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 is an obligate intracellular endosymbiont 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 ï 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 (5ô-ʁɸʊɸʁʁʊɸʊTCGAAGGGATAG, 5ô-AGCTTCGAGTGAACCCAATTC) [17] and *wsp* gene (81F 5ô-TGGTCCAATAAGTGATGAAGAAAC, 691R 5ô-

AAAAATTAAACGCTACTCCA) [18]. To confirm obtained results, each PCR was repeated twice.

Results. We have analyzed 203 imagos, 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 *Drosophila testacea* collected from the Chornobyl Exclusion Zone was positive for *Wolbachia*.

Table 1

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

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

N ***ï*** the total number of collected individuals; *n* ***ï*** number of individuals that had been tested; * *Wolbachia* positive sample

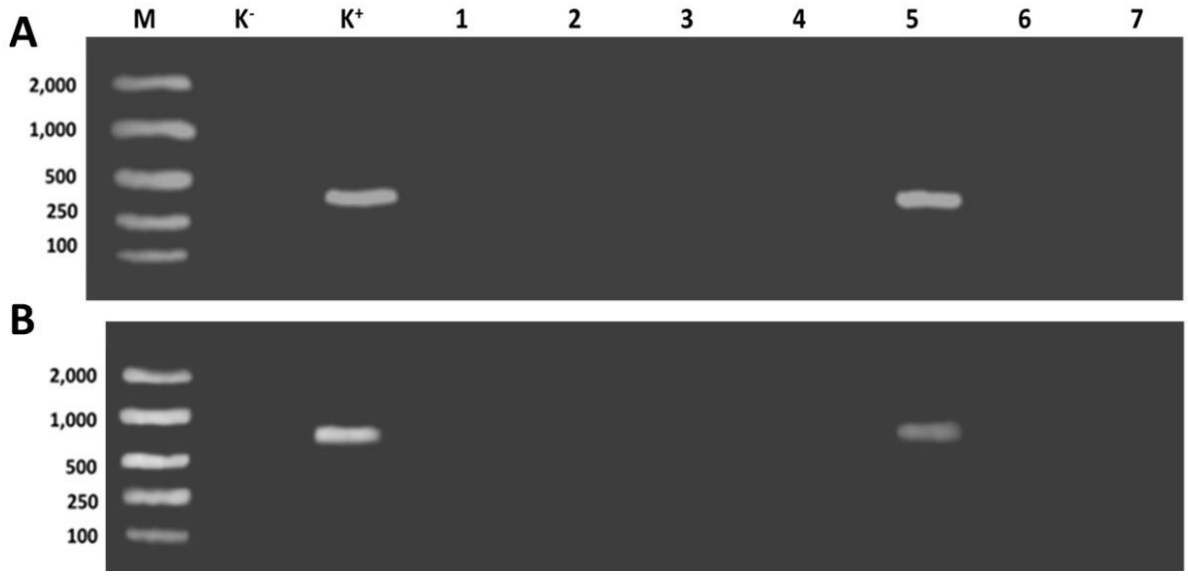

Fig. 1. Gel picture displaying PCR products of the 438 bp *16S rRNA* (A) and 632 bp *wsp* (B) gene fragments, used for detection of *Wolbachia* infection

Lanes: M ï 100 bp DNA Ladder ñNew England BioLabsò; K^- ï negative control; K^+ ï positive control *D. melanogaster*; 1 ï *D. testacea*; 2 ï *D. repleta*; 3 ï *D. hydei*; 4 ï *D. obscura*; 5 ï *D. subobscura*; 6 ï *D. busckii*; 7 ï *D. immigrans*.

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, characterised by a low prevalence of *Wolbachia* infection, as from more than 200 samples spanning across seven *Drosophila* species, just one individual of *D. testacea* was confirmed to be positive for *Wolbachia* infection. 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* (43ï 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 *DrosophilΘ* species examined in the present study. Therefore, *Wolbachia* infects only 3 out of 9 *Drosophila* species surveyed in Ukraine.

Acknowledgments. Authors thanks the staff of the Department of Biology at Mechnikov National University of Odesa, and the staff of the National Institute of Viticulture and Winemaking, National Academy of Agrarian Sciences of Ukraine, and Alexandra Protsenko and Mykhailo Lavrinienko for their invaluable help in material collection.

ɩ ɾ ɩɩ ɸʏɩ ɾ ɟ ʃ ʑ ʀ ʈ ɾ ɩ ɩ ɕ ɰ ʌ ɰ ʊ ɺ ɸɩ ɩ ɕ *WOLBACHIA* ɺ ɟ ʈ ʀ ʈ ʃ ɼ ɩ ʀ ʍ ɟ ʃ ɟ ʊ ʃ ɕ ʎ ɰ ʍ *DROSOPHILA* ɺ ʊ ʂ ʈ ɸɰ ɯ

***ʉ*. *ɺ* . *ʉʝ tɑɢɵ*[1], *ɟ* .*A*. *ʂ tsɘɵʝ dɮts*[1], *ɩ* .*ɺ* . *ɻ tstɑɵ*[1], *A*.*ɺ* . *ʃ ɵɘtɕ dʑ dɮts*[2], *ʉ*.*ɺ* . *ɼ ʝ dʒʜtsɘ*[1], *ʌ* . *ʃ ʝ fɱs tɕ fɾ*[3], *M*. *ɟ ɵfʂ lzɵlz*[3], *ɯA*. *ʂ tsɿʝ tɕ ɥ ʕɵ*[1]

[1] [illegible], 64, [illegible], 01601, [illegible]
[2] [illegible], 90014, [illegible]
[3] [illegible], E-08028, [illegible]

[illegible]

[illegible] Wolbachia [illegible] Drosophila, [illegible]. [illegible] Wolbachia [illegible] 203 [illegible] 7 [illegible] 7 [illegible]. [illegible] Wolbachia [illegible] Drosophila testacea, [illegible]. [illegible] Wolbachia [illegible] Drosophila melanogaster [illegible] Drosophila simulans [illegible] 3 [illegible] 9 [illegible].

[illegible]. Wolbachia, Drosophila, [illegible], D. testacea, [illegible].

[illegible] WOLBACHIA [illegible] DROSOPHILA [illegible]

[illegible].A. [illegible][1], A. [illegible][2], M. [illegible][3], [illegible].A. [illegible][1]

[1] [illegible], 64, [illegible], 01601, [illegible]
[2] [illegible], 90014, [illegible]
[3] [illegible], E-08028, [illegible]

[illegible]

[illegible] *Wolbachia* [illegible] *Drosophila*, [illegible] *Wolbachia* [illegible] 203 [illegible] 7 [illegible] 7 [illegible]. *Wolbachia* [illegible] *Drosophila testacea*, [illegible]. *Wolbachia* [illegible] *Drosophila melanogaster* [illegible] *Drosophila simulans* [illegible] 3 [illegible] 9 [illegible].

[illegible] *Wolbachia*, *Drosophila*, [illegible], *D. testacea*, [illegible].

1. Belousov AO, Kozeretskaia IA. Symbiotic bacteria, which modify reproduction processes of *Drosophila melanogaster*. Mikrobiol zh. 2011; 73(2):43ï52.
2. Mcneill MR, Richards NK, White JA, Laugraud A. Hidden arsenal: ȷndosymbionts in arthropods, their role and possible implications for biological control success. N. Z. Plant Prot. 2014; 67:204ï212.
3. Sazama EJ, Ouellette SP, Wesner JS. Bacterial endosymbionts are common among, but not necessarily within, insect species. Environmental Entomology. 2019; 48 (1):127ï133. https://doi.org/10.1093/ee/nvy188
4. Kriesner P, Conner WR, Weeks AR, Turelli M, Hoffmann AA. Persistence of a *Wolbachia* infection frequency cline in *Drosophila melanogaster* and the possible role of reproductive dormancy. Evolution (N Y). 2016; 70:979ï997.
5. Hamm CA, Begun DJ, Vo A, Smith CCR, Saelao P, Shaver AO, et al. *Wolbachia* do not live by reproductive manipulation alone: infection

polymorphism in *Drosophila suzukii* and *D. subpulchrella*. Mol Ecol. 2014; 23:4871ï 4885.

6. Bourtzis K, Nirgianaki A, Markakis G. and Savakis C. *Wolbachia* infection and cytoplasmic incompatibility in Drosophila species. Genetics. 1996; 144:1063ï 1073.
7. Mateos M, Castrezana SJ, Nankivell BJ, Estes AM, Markow TA, Moran NA. Heritable Endosymbionts of Drosophila. Genetics. 2006; 174(1):363ï 376. doi:10.1534/genetics.106.058818.
8. Lo N, Paraskevopoulos C, Bourtzis K, O'Neill SL, Werren JH, Bordenstein SR, Bandi C. (2007) Taxonomic status of the intracellular bacterium *Wolbachia pipientis*. Int J Syst Evol Microbiol**.** 2007; 57:654ï 657.
9. Weinert LW, Araujo-Jnr EV, Ahmed MZ, Welch JJ. The incidence of bacterial endosymbionts in terrestrial arthropods. Proc Biol Sci. 2015; 282(1807):20150249.
10. Haine ER, Pickup N J and Cook JM. Horizontal transmission of *Wolbachia* in a Drosophila community. Ecol. Entomol. 2005; 30:464ï 472. doi: 10.1111/j.0307-6946.2005.00715.x
11. Serga SV, Kozeretskaya IA. The puzzle of *Wolbachia* spreading out through natural populations of *Drosophila melanogaster*. Zhurnal obshchey biologii. 2013; 74 (2):99ï 111. Russian.
12. Serga S, Maistrenko O, Rozhok A, Mousseau T, Kozeretska I. Fecundity as one of possible factors contributing to the dominance of the *w*Mel genotype of *Wolbachia* in natural populations of *Drosophila melanogaster*. Symbiosis. 2014; 63(1):11ï 17. doi:10.1007/s13199-014-0283-1.
13. Serga S, Maistrenko O, Rozhok A, Mousseau T, Kozeretska I. Colonization of a temperate-zone region by the fruit fly *Drosophila simulans* (Diptera: Drosophilidae). Canadian Journal of Zoology. 2015; 93(10):799ï 804. doi: 10.1139/cjz-2015-0018.

14. Lavrinienko A, Kesäniemi J, Watts PC et al. First record of the invasive pest *Drosophila suzukii* in Ukraine indicates multiple sources of invasion. J Pest Sci. 2017; 90:421ï 429. doi: 10.1007/s10340-016-0810-3.
15. Bächli G, Vilela CR, Escher SA and Saura A. The Drosophilidae (Diptera) of Fennoscandia and Denmark. Netherlands, Leiden: Brill Publishing House. 2005. p. 1ï 362 (Fauna Entomologica Scandinavinca: Vol. 39).
16. Aljanabi S. Universal and rapid salt-extraction of high quality genomic DNA for PCR- based techniques. Nucleic Acids Res. 1997; 25(22):4692ï 4693. doi:10.1093/nar/25.22.4692
17. Oô Neill S, [et al,]. 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc Natl Acad Sci USA. 1992; 89(7):2699ï 2702.
18. Zhou W, Rousset F, Oô Neill SL. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. Proc R Soc Lond B Biol Sci. 1998; 265:509ï 515. doi: 10.1098/rspb.1998.0324.
19. Stahlhut J, Desjardins C, Clark M, Baldo L, Russell J, Werren J, and Jaenike J. The mushroom habitat as an ecological arena for global exchange of *Wolbachia*. Mol. Ecol. 2010; 19:1940ï 1952. doi: 10.1111/j.1365-294X.2010.04572.x.
20. Werren J, Jaenike J. *Wolbachia* and cytoplasmic incompatibility in mycophagous Drosophila and their relatives. Heredity. 1995; 75:320ï 326.
21. Jaenike J, Stahlhut JK, Boelio LM, Unckless RL. Association between *Wolbachia* and *Spiroplasma* within *Drosophila neotestacea*: an emerging symbiotic mutualism? Mol Ecol. 2010; 19(2):414ï 25. doi:10.1111/j.1365-294X.2009.04448.x.

ʃ lstecʃdʒ·Odzs: 19.12.2018 tɞ