Symbiosis (2021) 83:163–172
https://doi.org/10.1007/s13199-020-00743-3

Effects of *Wolbachia* infection on fitness-related traits in *Drosophila melanogaster*

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Received: 2 May 2020 / Accepted: 24 December 2020 / Published online: 5 January 2021

Abstract
Wolbachia is an intracellular symbiont that infects a large number of arthropod species, ensuring its success in populations by influencing host reproduction. The *w*Mel strain in *Drosophila melanogaster* does not cause any strong modifications of sexual reproduction. Consequently, it is not clear how the high infection rates of the bacterium in populations of this species are maintained. The *w*Mel strain is classified into two groups of genotypes - *w*Mel and *w*MelCS. The *w*Mel genotype is ubiquitous in populations, while *w*MelCS is rare. In this study, we analyzed fitness-related traits in isofemale lines from the unique natural population from Uman (Central Ukraine), in which we observed preservation of the rare *w*MelCS genotype despite the fluctuations of infection rates between years. We analyzed these effects of *Wolbachia* genotype and host genetic background on important fitness parameters such as sensitivity to cold and oxidative stress, female fecundity and lifespan. We found that, in the studied population, *Wolbachia* had an impact on fitness traits only in certain Drosophila genotypes. Positive effects were manifested in the alterations of fecundity, but at the cost of reduced lifespan and resistance to stress. Based on these findings, we conclude that the effect of bacteria on fitness and stress related traits is context-dependent and is modified by the host genotype, at least in the lines established from the Uman population.

Keywords *Wolbachia* · *Drosophila melanogaster* · Lifespan · Oxidative stress · Cold stress · Fecundity

1 Introduction

Microbiota is increasingly regarded as an important factor that contributes to the hosts' physiology (McFall-Ngai et al. 2013). In arthropods, infection with endosymbiotic bacteria, such as *Wolbachia*, is among the most important. *Wolbachia* are maternally inherited endosymbiotic bacteria that infect a variety of terrestrial arthropods (Hilgenboecker et al. 2008; Duron et al. 2008; Werren et al. 2008; Serga et al. 2019). Evolutionary success of bacteria in populations of the host species relies on the mode of influencing the reproduction of the host, such as cytoplasmic incompatibility (CI), male killing (MK), feminization of genetic males and induction of parthenogenesis (O'Neill 1998). Both the nature and intensity of reproductive manipulations depend on host genetics and *Wolbachia* strain (Braig et al. 1994; Veneti et al. 2012). In *Drosophila*, *Wolbachia* are known to cause the CI and MK reproductive phenotypes. It has been shown that the *w*Ri strain, which causes CI, spread rapidly in the populations of *Drosophila simulans* in California (Turelli and Hoffmann 1991, 1995) and in eastern Australia (Kriesner et al. 2013) with the current infection rate close to 100%. In *D. innubila,* the MK strain confers a selective advantage of about 5% and is maintained at 35% infection frequency in populations (Dyer and Jaenike 2004). However, the *Wolbachia* strains *w*Mel and *w*Suz have low to no effects on reproductive phenotypes with infection levels often lower than 100% (Hoffmann 1988; Solignac et al. 1994; Hoffmann et al. 1996; Hamm et al. 2014). CI variability, at least in the case of *D. melanogaster*,

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can be explained by "young brothers" and "grandmother" effects (Yamada et al. 2007; Layton et al. 2019). In cases with low levels of CI, maintenance of the infection in a population is explained by particular fitness benefits (Serga and Kozeretskaia 2013), although the reasons for the success of bacteria in natural populations infected with non-CI or non-MK strains are not always clearly identifiable (Charlat et al. 2004; Harcombe and Hoffmann 2004; Fry et al. 2004).

*w*Mel is the only strain presented in natural populations of *D. melanogaster* (Solignac et al. 1994; Verspoor and Haddrill 2011). This strain is classified into two groups of genotypes – *w*Mel and *w*MelCS (Riegler et al. 2005). The frequency of the genotypes varies across natural populations of *D. melanogaster* (Riegler et al. 2005; Serga et al. 2014; Bykov et al. 2019). Presently, flies infected with the *w*Mel genotype are predominant in most populations. *w*MelCS is a rather rare genotype, although there are populations in which it prevails, for example, in some populations of Portugal and Asia (Ilinsky and Zakharov 2007; Nunes et al. 2008). In addition, it has been shown that the *w*MelCS genotype is prevalent among laboratory lines collected before the 1950s, while the *w*Mel genotype was predominantly identified in the lines collected in the second half of the twentieth century (Riegler et al. 2005). Based on this fact, it was hypothesized that the *w*MelCS genotype was replaced worldwide with *w*Mel. The causes of the replacement of *w*MelCS with *w*Mel are not completely clear. According to one hypothesis, *w*MelCS is more costly for the host because of its higher titers in the host organism compared to *w*Mel (Chrostek et al. 2013). According to an alternative hypothesis, flies infected with *w*MelCS prefer colder environments than those infected with *w*Mel. This leads to a higher rate of development of flies with *w*Mel, which increases their fitness and increases the number of generations per year, eventually leading to a replacement (Truitt et al. 2019). However, there are populations where both rare (*w*MelCS-like) and common genotypes (*w*Mel-like) are present at relatively high frequencies, for example, in a population from Uman in Ukraine (Serga et al. 2014).

In the absence of high levels of CI and MK, the evolutionary success of *Wolbachia* is determined by the ratio of negative and positive effects on *D. melanogaster* fitness, as well as by imperfect maternal transmission (Kriesner et al. 2016). Fry et al., 2004 have shown that infection with *Wolbachia* leads to different fitness effects depending on the *D. melanogaster* line (Fry et al. 2004). In some lines, infection with *Wolbachia* leads to higher survival or fecundity, while in others to lower (Alexandrov et al. 2007; Maistrenko et al. 2015, 2016; Roshina et al. 2018; Capobianco et al. 2018). In particular, decreased lifespan has been reported from wild *Drosophila* strains collected in Russia (Roshina et al. 2018) and North America (Capobianco et al., 2018) and in inbred fly strains from Drosophila Genetic Reference Panel (Albertson et al., 2013), while an extend in lifespan has been observed in *Wolbachia*-positive laboratory lines (Alexandrov et al. 2007). The strain of bacteria can also be an important factor, but most of these studies did not perform genotyping of *Wolbachia*. *w*MelCS *Wolbachia* transferred from *D. melanogaster* via microinjection into *D. simulans* caused a reduction of lifespan and fecundity (Martinez et al. 2015). *Wolbachia* is also able to affect host sensitivity to physiological stress conditions (Brownlie et al. 2009; Wang et al. 2012; Albertson et al. 2013; Gruntenko et al. 2017), particularly oxidative stress (Wong et al. 2015; Capobianco et al. 2018) and viral infection (Hedges et al. 2008; Teixeira et al. 2008). The effect of the bacteria on the stress response has, however, not been detected in all lines and appears to depended on the flies' genetic background (Capobianco et al. 2018). For example, in lines from Australian natural populations of *D. melanogaster Wolbachia* did not influence adult starvation resistance and also had no effect on adult heat resistance (Harcombe and Hoffmann 2004). However, in inbred lines from North Carolina, removing *Wolbachia* with tetracycline induced differential starvation survival (Albertson et al. 2013).

To investigate the effects of *Wolbachia* on fitness traits, it is important to obtain genetically identical lines that are infected and uninfected with the bacterium. A number of approaches are used for this, such as antibiotic or temperature treatments (Li et al. 2014), as well as injection of bacterial strains to non-natural hosts (Martinez et al. 2015). The most effective antibiotics are tetracycline and rifampicin (Li et al. 2014). Antibiotic treatment allows to quickly and efficiently obtain genetically identical lines infected and uninfected with *Wolbachia*. One problem is that the antibiotic itself can affect fitness traits (O'Shea and Singh 2015). In addition, broad spectrum antibiotics can affect the composition of the microbiota in general. The alternative approach is based on obtaining lines with the same genotypes infected with different *Wolbachia* strains by injection of bacteria (Martinez et al. 2015). In this case, the microbiome of the line is not disturbed, however, *Wolbachia* is introduced into a new genotype, which can also significantly affect fitness traits.

In this study, we analyzed fitness and stress related traits in isofemale lines from a unique natural population from Uman, in which persistence of the *w*MelCS genotype is observed from year to year with varying infection rates. We analyzed the effects of *Wolbachia* on important fitness parameters such as sensitivity to cold and oxidative stress, female fecundity and lifespan, depending on the bacterial genotype and on the host *Drosophila* genetic background. We found that, in the studied population, *Wolbachia* impacted fitness traits only in certain Drosophila genotypes. Positive effects were manifested in alterations of reproductive traits, but at the cost of reduced lifespan and lower resistance to stress. Based on these findings, we conclude that the effect of the bacteria on fitness and stress related traits depends on the host

genetic background, and not on the *Wolbachia* genotype, at least in the studied Uman population.

2 Materials and methods

Drosophila lines We used 6 isofemale lines of *D. melanogaster* (*Um59, Um8, Um16, Um15, Um25, Um37*) established from flies collected in 2012 in an apple garden near Uman, N 48°45′45.26“, E 30°14’38.97” (Serga et al. 2014). We also used laboratory lines *Canton-S* (provided by Lyudmila Zakharenko, Novosibirsk, Russia) and *Oregon-R* (Bloomington Drosophila Stock Center, USA). Lines *Um59, Um8,* and *Um16* were infected with the *w*Mel genotype of *Wolbachia*, lines *Um25* and *Canton-S* were infected with *w*MelCS, and *Oregon-R, Um15* and *Um37* were not initially infected with *Wolbachia*. All isofemale lines had been cultivated in the laboratory for 3 years before experiments started.

To create genetically similar infected and uninfected fly lines from a single stock, we used an antibiotic treatment. We created tetracycline-treated (T) lines by adding 0.25 mg/ml of tetracycline to the cultivation medium (6 g agar-agar, 50 g semolina, 80 g yeast, 50 g sugar and 2 mL propionic acid per 1 L of water). All flies were reared for two generations on media with antibiotics and four generations on media without antibiotics to mitigate their effects (Fry et al., 2004). After treatment with antibiotics, all lines were tested for presence/absence of *Wolbachia* by PCR. Since antibiotics can potentially affect fly fitness, we also included lines initially uninfected with *Wolbachia* to account for the potential effect of tetracycline.

***Wolbachia* detection and genotyping** DNA was extracted from 20 whole adult flies of each strain by the high-salt method (Aljanabi and Martinez 1997). *Wolbachia* infection was tested by PCR using a published set of primers to bacterial 16S rDNA (O’Neill et al. 1992) and the *wsp* gene (Zhou et al. 1998). Each PCR was repeated twice. *Wolbachia* genotype was identified by the number of the minisatellite repeats VNTR-141, VNTR-105 and the presence of the insertion sequence IS5 in the loci WD0516/7 and WD1310 of the *Wolbachia* genome as described in Riegler et al. (2005).

Survival Fly survival was estimated for lines *Um59, Um8, Um16, Um25, Um37, Canton-S* and *Oregon-R* treated and not treated with tetracycline. 1–3 day old male flies were placed into vials (14 cm in length and 2 cm in diameter) with the standard medium (10 flies per vial) and were reared at 24–25°C. Live flies were counted every 3 days and transferred into vials with a fresh medium. 100 flies were used for measuring lifespan in each line. Maximum lifespan was determined as the day when no flies remained alive.

Fecundity We estimated fecundity for all tetracycline-treated and intact lines except *Um16* and *Oregon-R*. For measuring fecundity, newly eclosed flies (mixed males and females) were kept up to 7 days of age in 200 mL bottles (50–60 flies per line). For each line, we selected 15–20 flies that were 5 days old and placed them in separate vials (10 cm length and 5 cm diameter) at 25°C. The flies were then removed from the vials after 22 h and the number of eggs was counted in each vial.

Cold tolerance Cold tolerance was estimated via the chill-coma recovery time approach in all tetracycline-treated and intact lines from Uman and *Canton-S*. For this test, 10 males of each line were subjected to temperature stress at −9 °C for 45 s. After that, the flies were placed in 28 °C and the time in seconds until the beginning of the first movement was determined. Cold stress test was performed for male flies aged 3 and 21 days.

Oxidative stress Oxidative stress tolerance was estimated for all the lines (except *Oregon-R*) treated and not treated with tetracycline according to Lander’s method (Jünger et al. 2003). Hydrogen peroxide was used as a prooxidant (5% solution). For positive control, we used a 10% sucrose solution. One hundred 3 days old males were placed in test tubes with the agarose medium (10 individuals in each) and treated with 200 μl of 5% H_2O_2 in 10% sucrose added to the filter. Every two days, flies were transferred into vials containing fresh medium. Sensitivity to oxidative stress was determined by the survival rate at 96 h after the exposure.

Statistical analysis Kolmogorov-Smirnov test was used to verify the nature of the distribution of the lifespan data. Mann-Whitney test was used to test for differences in lifespan and fecundity. For cold stress tolerance, we used ANOVA followed by the post-hoc Tukey HSD test for pairwise comparisons (Tukey 1949). Differences in tolerance to oxidative stress were analyzed with Fisher’s exact test (Fisher 1922). Differences among the host genotypes were estimated by comparing tetracycline treated lines. All statistical analyses were performed using R v.3.4.4 (R Core Team 2018). The raw data for statistical analysis is available at https://github.com/omaistrenko/WolbachiaPhenotypesSymbiosis.

3 Results

Survival Lifespan analysis of the host genotypes from the Uman population revealed variability (Supplementary Table 1), whereby the *Um25* line was longer lived compared to *Um59* and *Um37* (Mann-Whitney test, p=0.009 and p=0.013, Benjamin-Hochberg correction). Lifespan was shorter in the *Canton-S* and *Oregon-R* laboratory lines compared to most of the other lines established

from the Uman population (Mann-Whitney test, $p<0.05$, Benjamin-Hochberg correction).

Two lines (*Um8/w*Mel and *Canton-S/w*MelCS) had significantly longer lifespans under tetracycline treatment (Table 1, Fig. 1). These lines were infected with different *Wolbachia* genotypes. We compared the lifespan of the lines infected with the *w*Mel *Wolbachia* genotype (*Um8, Um16* and *Um59*) with that of the lines with the *w*MelCS genotype (*Um25* and *Canton-S*). Lines *Um8* and *Um59* infected with *w*Mel demonstrated a shorter lifespan compared to *Um25* infected with *w*MelCS (Mann-Whitney test, p <0.00001 and p=0.00024). The lifespan of *Canton-S/w*MelCS laboratory line was shorter compared to *Um59/w*Mel and *Um16/w*Mel (Mann-Whitney test, p=0.00288 and p <0.00001) and did not differ from *Um8/w*Mel (Table 1; Mann-Whitney test, p=0.0601). In other words, host genotype is a more important factor influencing lifespan compared to the presence of *Wolbachia* and its genotype.

Fecundity We did not observed differences in fecundity in lines established from the Uman population (Mann-Whitney test, $p>0.05$, Benjamin-Hochberg correction). However, the laboratory line *Canton-S* had lower fecundity when compared to other lines (except Um37) (Mann-Whitney test, $p<0.05$, Benjamin-Hochberg correction) (Supplementary Table 2).

We analyzed flies' fecundity before and after treatment with tetracycline. In most lines, we did not find differences between tetracycline-treated and untreated flies (Mann-Whitney test, $p> 0.05$) (Fig. 2). In *Um8/w*Mel and *Canton-S/w*MelCS, which were infected with different *Wolbachia* genotypes, the number of eggs laid after tetracycline treatment significantly decreased from (Mann-Whitney test, p=0.00228 and p=0.00096 respectively). Interestingly, *Wolbachia* increases fecundity in the same lines in which it reduces life expectancy.

Cold tolerance We observed an effect of *Wolbachia* on chill coma recovery time only in line *Um8/w*Mel (Fig. 3.). This effect was only detectable in 21 days old flies. Flies infected with *Wolbachia* recovered slower from a chill coma (TukeyHSD, p=0.0001). The obtained results suggest that *Wolbachia* could only affect cold tolerance in certain host genotypes. However, comparison between host genotypes from different lines (after tetracycline treatment) did not show any differences in cold recovery (Supplementary Table 3).

Oxidative stress We did not observe any effect of the host genotype on oxidative stress response. Only the *Um15* line had significantly lower oxidative stress resistance in presence of tetracycline among the studied lines (Fisher's exact test, p<0.05, Benjamin-Hochberg correction) (Supplementary Table 4).

We observed putatively *Wolbachia*-induced differences in oxidative stress tolerance only in line *Um59/w*Mel, whereby flies treated with tetracycline had higher tolerance to oxidative stress (Fisher's exact test, p= 0.001, Benjamin-Hochberg correction, Table 2). The effect of *Wolbachia* on oxidative stress is likely to be insignificant and/or dependent on host genetic background rather than *Wolbachia* genotype and requires larger datasets to obtain conclusive results.

Combined effects of *Wolbachia* on host fitness-related traits In the present study, we analyzed fitness-related traits of the same fly lines, which allows us to draw conclusions about trade-offs between phenotypes and presence of *Wolbachia* (Table 3). Both studied genotypes of *Wolbachia* (*w*Mel and *w*MelCS) had an effect on the host's phenotype. But for the *w*Mel genotype of *Wolbachia*, we observed alterations in all the studied phenotypes of the host: lifespan, fecundity, cold and oxidative stress response. The strongest effect of the bacteria was observed in the *Um8/w*Mel line, in which *Wolbachia*

Table 1 Lifespan statistics for tetracycline treated and untreated fly lines

Fly line	Tetracycline treatment	Initial *Wolbachia* status	Median life span, days	Max life span, days	Kolmogorov-Smirnov test, p value	z-score	p value
Um16	–	*w*Mel	32	55	0.0177	−0.837	0.4009
	+		31	51	0.0153		
Um59	–	*w*Mel	30	51	0.0074	−1.603	0.1096
	+		32	55	0.0518		
Um8	–	*w*Mel	21	45	0.0064	−4.348	<0.00001
	+		36	55	0.0148		
Um25	–	*w*MelCS	37	52	<0,00001	−0.829	0.40654
	+		37	52	0.00015		
Um 37	–	Not infected	30	51	0.0114	−1.528	0.12602
	+		32	55	0.0468		
Canton-S	–	*w*MelCS	21	45	0.0077	−2.588	0.0096
	+		24	42	0.0195		
Oregon-R	–	Not infected	23	44	0.0356	0.211	0.83366
	+		28	40	0.0223		

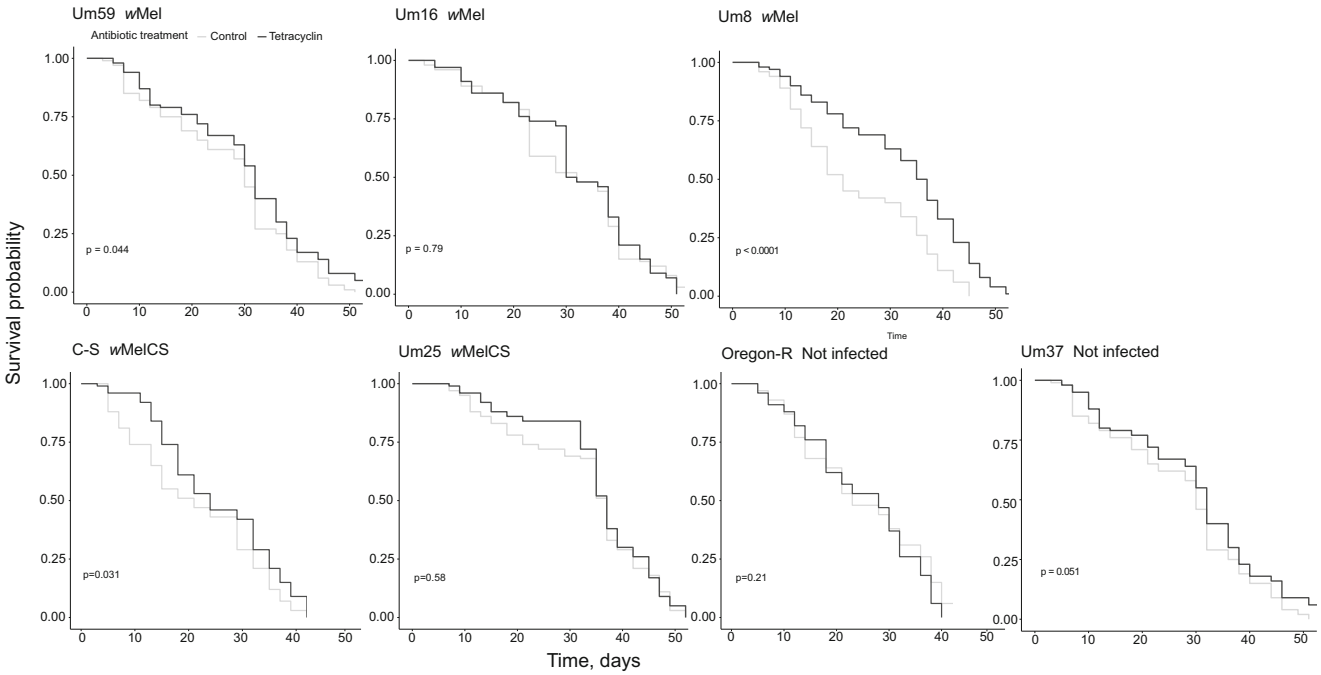

Fig. 1 Survival curves for all fly lines. *Wolbachia* significantly lowered survival in the *Um8* and *Canton-S* lines (*P*-values obtained from the "survfit" function that fits the Kaplan-Meier regression model)

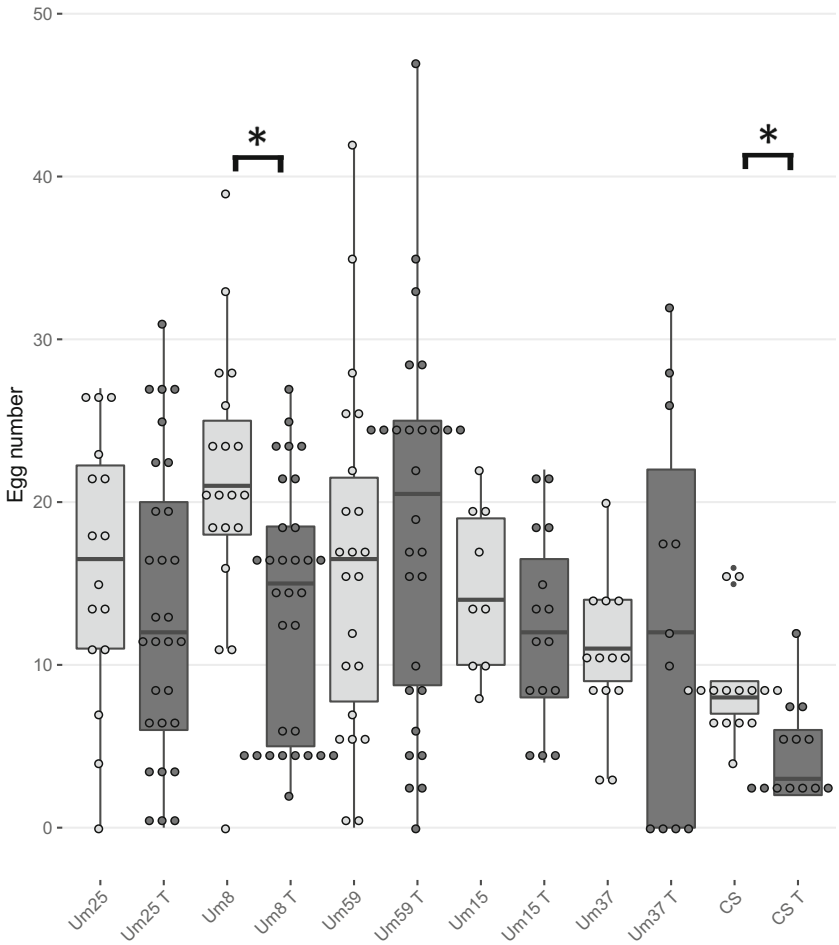

Fig. 2 Fecundity of all analyzed fly lines (treated with tetracycline (T) and untreated)). *Um25* and *Canton-S* infected with the *w*MelCS *Wolbachia* genotype, *Um8* and *Um 59* infected with the *w*Mel genotype, *Um15* and *Um37* not initially infected

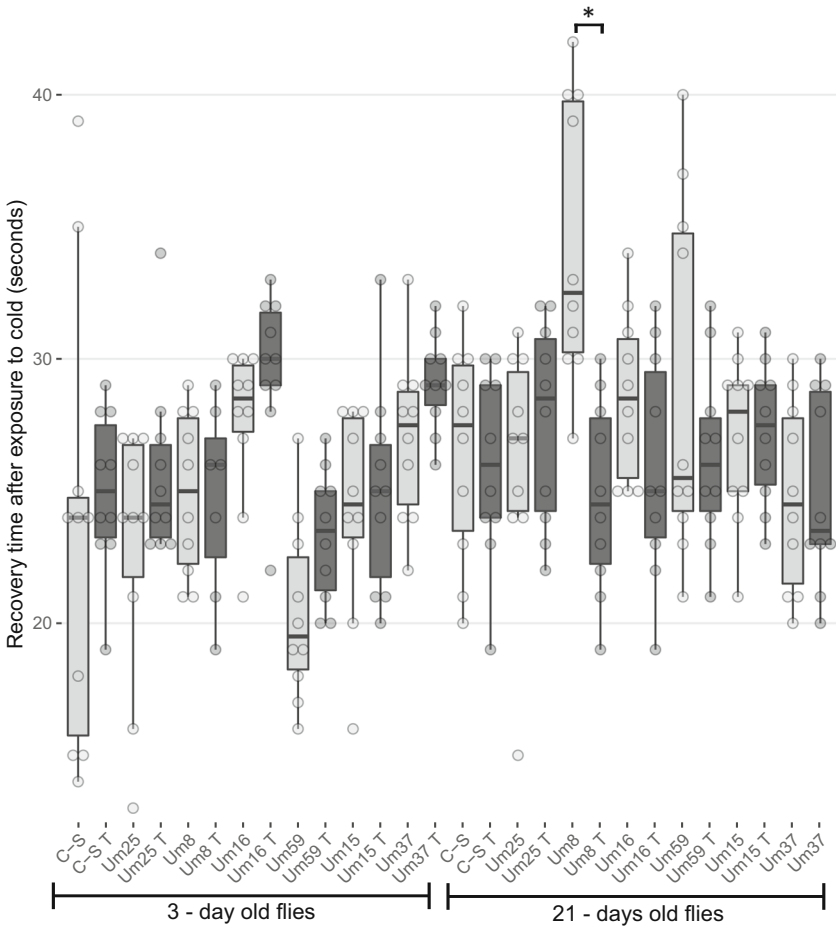

Fig. 3 Chill coma recovery time after exposure to extreme cold temperature of males in all analyzed lines (treated with tetracycline, T, and untreated). *Canton-S*, *Um25* infected with the *w*MelCS *Wolbachia* genotype, *Um8, Um16* and *Um59* infected with the *w*Mel genotype, *Um15* and *Um37* not initially infected

significantly increased fecundity, but at the cost of a lower life expectancy and sensitivity to cold stress. The effect of the bacteria under the influence of oxidative stress was observed only for a single line, *Um59/w*Mel. The study also included lines (*Um15, Um37* and *Oregon-R*) that were not initially infected with the bacterium but were also treated with tetracycline. We did not observe any effect of the antibiotic treatment on these lines, indicating that antibiotic is unlikely to be a confounding factor in this study. The obtained data indicate that the effect of *Wolbachia* depends more on the Drosophila genotype rather than the genotype of *Wolbachia* itself.

4 Discussion

In this study, we evaluated effects of *Wolbachia* on various life history traits (fecundity, lifespan, cold and oxidative stress) in *D. melanogaster* isofemale lines established from a unique Uman population that is stably infected by the cosmopolitan *w*Mel genotype and the rare *w*MelCS genotype of *Wolbachia* (Serga et al. 2014). The infection frequency varied during the many years of monitoring (Ilinsky and Zakharov 2007; Serga et al. 2014). So, this population is likely a good model to investigate the mechanisms underlying the greater

Table 2 Comparisons of tetracycline treated and untreated flies in the oxidative stress assay, F-test (n=100)

Fly line	Type of infection	% survival, tetracycline treated fly lines	% survival, untreated fly lines	F-test p value
Canton-S	*w*MelCS	13	19	0.071
Um15	uninfected	3	11	0.049
Um16	*w*Mel	24	12	0.095
Um25	*w*MelCS	13	10	0.514
Um37	uninfected	39	31	0.483
Um59	*w*Mel	26	6	0.001
Um8	*w*Mel	4	22	0.082

Table 3 Effects of *Wolbachia* on different fitness-related traits

Fly line	Type of infection	Lifespan	Fecundity	Cold stress	Oxidative stress
Canton-S	*w*MelCS	decreased	increased	not affected	not affected
Oregon-R	uninfected	not affected	n/a	n/a	n/a
Um16	*w*Mel	not affected	n/a	not affected	not affected
Um8	*w*Mel	decreased	increased	decreased	not affected
Um59	*w*Mel	not affected	not affected	not affected	decreased
Um25	*w*MelCS	not affected	not affected	not affected	not affected
Um37	uninfected	not affected	not affected	not affected	not affected
Um15	uninfected	n/a		not affected	not affected

n/a - not assayed

overall success of the *w*Mel genotype compared to *w*MelCS worldwide, whereby the former genotype has replaced the latter in most fly populations, but still co-exists with it in some. In the absence of significant levels of CI or other reproductive manipulation phenotypes, the success of the bacteria of a certain strain in *Drosophila* populations is thought to be determined by the transmission rate and fitness benefits (Gundel et al. 2011). Transmission fidelity does not differ between the *w*Mel and *w*MelCS genotypes and, depending on the fly genotype, reaches 90–100% (Serga et al. 2014). We analyzed the impact of *Wolbachia* on fitness related traits, such as fecundity, lifespan, survival under the influence of cold and oxidative stress response. The effects of *Wolbachia* on *Drosophila* fitness have been investigated repeatedly in multiple studies before, but many studies had conflicting results (Fry et al. 2004; Alexandrov et al. 2007; Roshina et al. 2018; Capobianco et al. 2018). In this paper, we analyzed several fitness related traits in the same lines. We found moderate effects of *Wolbachia* infection on fitness that depended on the fly's genotype rather than the *Wolbachia* genotype.

It has been previously shown that *Wolbachia* infection can decrease the lifespan of *D. melanogaster* (Min and Benzer 1997). Significant shortening of lifespan was observed as a result of infection with the *w*MelPop strain and moderate for the *w*MelCS genotype (Chrostek et al. 2013). Pathogenic genotypes of *Wolbachia* are likely to stimulate the immune system. It has been shown previously that bacteria can overactivate the immune system which in turn is associated with decreased lifespan (Libert et al. 2006). Moreover, infected flies showed a decrease in lifespan compared to tetracycline-treated wild Drosophila strains collected in Russia (Roshina et al. 2018), North America (Capobianco et al., 2018), and inbred lines from *Drosophila* Genetic Reference Panel (Albertson et al. 2013), however, the bacteria were not genotyped in these studies. In our study, we show that in two lines infected with two different *Wolbachia* genotypes, there is also a decline in lifespan in infected individuals compared to those from which the bacterium was removed using antibiotics treatment. At the same time, Albertson et al. (2013) have shown that a similar effect of lifespan increase after treatment with tetracycline is observed in both *Wolbachia*-infected and uninfected lines. This finding indicates that presence of other bacteria might be affecting lifespan (Albertson et al. 2013).

The increased fecundity of one *w*Mel-infected line from the Uman population and a *w*MelCS-infected laboratory line observed in the present study is consistent with our previous observations (Serga et al. 2014) and contradicts findings from Australian populations, where the effect of *Wolbachia* on fecundity was not detected (Hoffmann et al. 1994). Similarly to our previous results, we did not observe any effect of the *w*MelCS genotype from the Uman population on fecundity (Serga et al. 2014). It is possible, that the *w*Mel genotype is more capable of affecting the phenotype of the host and consequently promoting itself in the population. In our study, the *w*Mel genotype of *Wolbachia* affected all of the studied phenotypic traits in the host: lifespan, fecundity, and cold and oxidative stress responses. Interestingly, higher fecundity and shorter lifespans in our study were observed in the same lines infected with the *w*Mel genotype. Reproductive activity is known to be one of the key factors that affect life expectancy in *D. melanogaster* (Piper and Partridge 2018). Increased reproductive activity is usually associated with reduced lifespan (Partridge and Harvey 1988; Flatt 2011). According to the "cost of reproduction" concept, a trade-off between longevity and reproduction may be likely explained by a reallocation of nutritional and other resources from somatic maintenance to reproduction (Fowler and Partridge 1989; Adler et al. 2013). So, it may be assumed that *Wolbachia* infection can promote early reproductive success at the cost of lifespan; these phenotype alterations are dependent on the host genotype. Thus, the interactions between the host and *Wolbachia* genotypes may potentially lead to context-dependent fitness effects that cause incomplete replacement of *w*MelCS by the *w*Mel genotype in natural population.

Another explanation for the success of bacteria in a natural population is modulation of the response to stress factors in infected individuals. Temperature is one of the most important environmental abiotic factors that affect the physiology and

life history traits. Response to temperature can be affected by host-symbiont interactions (Corbin et al. 2017). Firstly, temperature affects the *Wolbachia* titers in the host organism and its transmission rate (Ross et al. 2017). Secondly, *Wolbachia* can influence *Drosophila* thermal preferences. *w*Mel/*w*MelsCS-infected flies prefer warmer conditions than uninfected flies (Truitt et al. 2019). Thirdly, the effect of *Wolbachia* on the timing of recovery from a chill coma has been shown for inbred lines earlier (Albertson et al. 2013). In our study, we found that the *w*Mel-infected *Um8* line demonstrated poorer cold tolerance. However, this effect likely depended on the age of the flies and on the genetic background. In an earlier study, the influence was inconsistent and the presence of *Wolbachia* either increased or decreased the recovery rate depending on the *Drosophila* line (Albertson et al. 2013). These findings together with our work suggest that cold recovery potentially depends on the interactions between host genetic background and *Wolbachia*.

Oxidative stress is another important factor that substantially influences aging and longevity. *Wolbachia* is known to induce excess ROS and, as a result, higher superoxide dismutase activity (Brennan et al. 2012). In our study, tolerance to external oxidative stress did not depend significantly on the *Wolbachia* genotype. Only the *Um59* line, originally infected with the *w*Mel genotype, had higher oxidative stress tolerance after tetracycline treatment. So, for this line, *Wolbachia* might have had a negative effect on the oxidative stress tolerance.

In this work, we used an approach in which fitness traits in the *Wolbachia*-host system can be investigated without transferring *Wolbachia* to the same fly genotype by using tetracycline antibiotic treatment. The advantage of this approach is the preservation of the natural interaction between *Wolbachia* and its host. However, tetracycline is a broad-spectrum antibiotic and can affect the composition of microbiota, as well as it can have negative effects on fitness traits (Li et al. 2014; O'Shea and Singh 2015). After antibiotic treatment, we conducted experiments on the 4th generation of files and we did not observe any effects of antibiotics on control lines that were initially not infected with *Wolbachia*.

Based on the results of this study and previous publications, we conclude that *Wolbachia* infection can cause both deleterious and beneficial effects on different fitness components in *Drosophila*, and these effects dependent on the host genetics rather than *Wolbachia* genotype. Moreover, the effects are not present in all fruit fly lines. Beneficial effects are often manifested in improved reproduction, which however likely comes at the cost of shortened lifespan and lower resistance to stress. Replacement of the supposedly more deleterious *Wolbachia* isolate *w*MelCS with the more neutral/beneficial *w*Mel appears to be in line with the overall directionality in the evolution of host-symbiont relationships from parasitic toward more neutral/mutualistic interactions.

In conclusion, we show that *Wolbachia* may affect fitness related traits in *Drosophila*, such as fecundity, lifespan and stress tolerance. Further analysis is required of fruit fly lines originating from multiple populations to disentangle the effects of *D. melanogaster* and *Wolbachia* genotypes on the fitness of this host-symbiont system.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13199-020-00743-3.

Acknowledgments The authors thank student of Ivan Franko National University of Lviv Ivanna Shushko for help with cold and oxidative stress experiments and Vadym Shulga Kyiv Scientific Research Forensic Center of the Ministry of Internal Affairs of Ukraine for help with the fecundity assay. We thank to the anonymous reviewer for a thorough review of the work. We thank Dr. A. Rozhok, University of Colorado School of Medicine for a preliminary revision of the manuscript.

Authors' contributions SS and IK conceived of the project design; SS and NM performed the research; OM, SS and AV analyzed the data; SS and OM wrote the manuscript with significant contributions from AV and critical input from IK.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability All data generated or analyzed in this study are included in the article.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval The study complies with all applicable international, national, and institutional guidelines for the care and use of animals in research.

Consent to participate Not applicable.

Consent for publication Not applicable.

Code availability Not applicable

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