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DYNAMICS OF FREQUENCIES OF *WOLBACHIA* GENOTYPES IN *DROSOPHILA MELANOGASTER* POPULATION FROM UMAN' UNDER INFLUENCE OF CLIMATE FACTORS

Summary

Aim. *To investigate the potential influence of climate parameters on the rate of Wolbachia genotypes frequencies in the population of D. melanogaster from Uman' for the last seven years.* ***Methods.*** *We have surveyed Wolbachia infection status for isofemale lines from Uman' collected during summer – fall 2013 and 2015-2017 using polymerase chain reaction (PCR) with primers specific to the 16S rRNA and wsp (Wolbachia surface protein) genes. To determine wMel and wMelCS genotypes of Wolbachia, we have conducted PCR using polymorphic markers for the presence of the insertion sequence IS5 in WD1310 locus and the copy number of minisatellite repeats in VNTR-141 locus. Infection status of flies for 2011, 2012 and 2014 years was included from our previous studies. Data of climate factors (average seasonal temperature, dew point, and precipitation) for winter and summer of each year were obtained from weather database. Statistical analysis of Wolbachia genotypes-climate factors interaction was carried out in R version 3.5.0 using multiple linear model regression.* ***Results.*** *We have observed the decline of Wolbachia presence in Uman' locality and prevalence of wMel bacteria variant. Moreover, our study documented the low persistence of rare wMelCS genotype through seven years in Uman'. wMelCS frequency has been driven by the combined influence of the average temperature and humidity in summer (p = 0.03662, R^2 = 0.9995).* ***Conclusions.*** *The climate variability affects frequency of wMelCS genotype of Wolbachia in the D. melanogaster natural population from Uman'.*

Key words: Drosophila melanogaster, Wolbachia, wMelCS, endosymbiont, climate factors, Uman', Ukraine.

Introduction

Endosymbiont *Wolbachia* is maternally inherited bacteria that are carried by a variety of terrestrial arthropods altering their reproduction throughout reproductive parasitism. The most frequent deleterious effect observed in a large fraction of host species is cytoplasmic incompatibility (CI) [27]. Despite negative impact, bacteria can also provide fitness advantages for the host such as viral resistance

[21], nutrient supply [2], higher fecundity [20] and adaptation under environmental stress [17].

The endosymbiont-host association can be influenced by climate changes such as fluctuation in temperature, humidity, precipitation, and weather phenomena. It was shown that presence of the endosymbionts such as *Curculioniphilus, Sodalis, Serratia, Wolbachia, Rickettsia,* and *Spiroplasma* in chestnut weevils is modulated with ecological factors [23]. *Wolbachia* has persistent clinal distribution among the natural population of *Drosophila melanogaster* in Australia [13]. It has been studied that *Wolbachia* is vulnerable to exposure to the higher temperature. Therefore, the higher temperature can reduce bacteria's negative impacts on hosts [10]. Frequency of bacteria is reduced under cold conditions and is rapidly increased after diapause in the parasitic wasp [18]. Hence, the environmental impact can modulate titers of endosymbiont and affect the selection pressure within the bacteria-host association. Recently, it has been shown that *Wolbachia* frequency correlates with longitude, altitude, and annual mean temperature in mites [26]. On the contrary, for ladybirds, hot climate does not affect the distribution of *Wolbachia* and their effects on the host [3].

Wolbachia has high distribution among *Drosophila*-group species, despite the weak cytoplasmic incompatibility in *w*Mel strain in *D. melanogaster* [8], *w*Au in *D. simulans* [9], *w*Suz in *D. suzukii* [7] and neutral strain in *D. mauritian* [5]. Low CI and inconsistent weak fitness effects cannot explain high frequencies of *Wolbachia* in host populations. Understanding of geographic variation in infection rates and its causes within species is also still limited. The fitness cost of host-*Wolbachia* interactions may vary due to abiotic factors and genetic backgrounds of host and symbionts.

Wolbachia is highly widespread in natural populations of *Drosophila melanogaster.* It is known that endosymbiont does not instigate any severe effects on a reproductive system of *D. melanogaster* and can provide fitness advantage for the host [4]. There are a few strains that infect *D. melanogaste*r in nature, *w*Mel is predominant among them. Nowadays, *w*MelCS is rare in nature [15]. Also, there is evidence of clinal variation of the spread of *w*Mel genotype in Australia, indicating that abundance of it declines from high to low latitudes [13]. Nevertheless, European populations have stable infection frequencies of *Wolbachia* from year to year [20] and, as it was shown previously, there is no clinal distribution of infection, at least in Eastern Europe [11]. Another aspect that should be considered, is that the European population of *D. melanogaster* was established much earlier than those ones in North America and Australia, about 10000–15000 years ago [13]. Thus, reproductive dormancy of flies from Europe during the winter period may differ reproductive dormancy from temperate climate of the rest world. There are studies that indicate that *Wolbachia*-infected flies prefer cooler conditions. Furthermore, *w*Mel infected flies, contrary to *w*MelCS infected, have a preference to warmer conditions, similar to which that uninfected flies prefer [1, 23]. Hence, we conclude that absence of clinal distribution of bacteria in European populations of *D. melanogaster* might be linked with other geographic factors such as local oscillation of climate in the area.

Uman' has a moderate continental climate with mild winters and warm

summers. Also, *w*MelCS strain has been detected in the 80s in this locality. We have collected the data on infection dynamics for Uman' population of *D. melanogaster* and climate factors for past 7 years. Our aim was to analyze the environmental effects on frequencies of *Wolbachia* genotypes in nature, particularly for infection in Uman' population of *D. melanogaster.*

Materials and methods

We collected *D. melanogaster* at the fruit orchards near local juice factory in Uman' (48°45'45.26"N 30°14'38.97"E) where *w*MelCS variant was detected in the 80s [11]. Flies were collected each fall (September-October) during 2013 and 2015–2017 years using active capture by an insect net and bait that contained apple pomace. The established isofemale lines [12] were used for further analysis. The flies were reared at 25 ± 1 and 70–80% relative humidity, were fed on a standard semolina-agar diet with yeast granules (6 g agar, 15 g yeast granules, 50 g sugar, 55 g semolina, 1 L of water). Propionic acid was used as an antifungal agent (4 mL propionic acid per 1 L medium).

Whole-bodies of 10–12 adult flies of each isofemale line were used for isolation of total genomic DNA by the salting-out method. To define *Wolbachia* infection-status, we surveyed the samples by PCR method using the set of primers to bacterial 16S rRNA [16] and *wsp* genes [25]. The genotypes of *Wolbachia* were determined via PCR using polymorphic markers for the presence of the insertion sequence IS5 in WD1310 locus (IS5 is presented in *w*MelCS) and the copy number of minisatellite repeats in VNTR-141 locus (6 and 7 copies in *w*MelCS and *w*Mel, respectively) [19]. PCR amplicons were visualized on 2% agarose gel.

Flies go through dormancy stage during winter and suffer consequent bottleneck effect. Population reaches its maximum size in summer. Therefore, we chose the climate parameters of winter (January and February only were included as winter months of the particular year) and summer season as potential factors that influence infection dynamics of both *w*Mel and *w*MelsCS strains. Climate data were obtained from www.wunderground.com and www.geographic.org (table 1). For the statistical analysis of data, multiple linear model regression was applied. Average seasonal temperature (TA), humidity (dew point – DA), precipitation (PA) were used as independent variables and infection level was used as dependent variable. ANOVA was used to detect significant factors affecting genotype frequencies. The normality and stability of variables were assessed using appropriate statistical tests (Studentized Breush-Pagan, Durbin-Watson test). For infection rates, Clopper-Pearson's confidence interval method was applied. All statistical analyses were carried out in R version 3.5.0 using *lmtest* package [22].

Results

We analyzed 210 isofemale lines from Uman'. Our results (data for 2013, 2015–2017 yr.) in combination with data from Serga et al. [20] (for 2011–2012 yr.) and Gora et al. [6] (for 2014 yr.) of *Wolbachia* screening (fig. 1) indicate the tendency for decrease of infection rate for past 7 years in Uman' (fig. 2) and an explicit prevalence of the genotype *w*Mel over *w*MelCS.

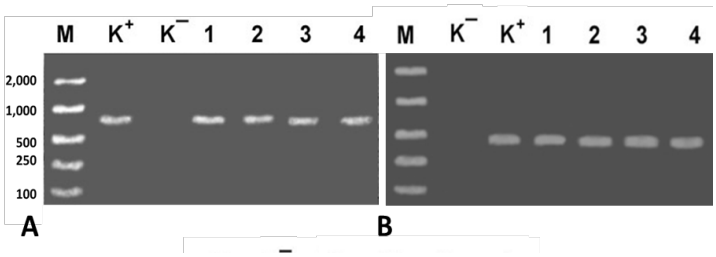

Fig. 1. Electrophoregrams of infection detection (A, B):
M – 100 bp DNA Ladder, Thermo Fisher Scientific; K^+ – positive control from total DNA of Canton-S strain; K^- – negative control showing no infection; 1–4 – amplicons of infected isofemale lines; A – *wsp* gene (size of band – 632 bp); B – *16S* RNA (438 bp)

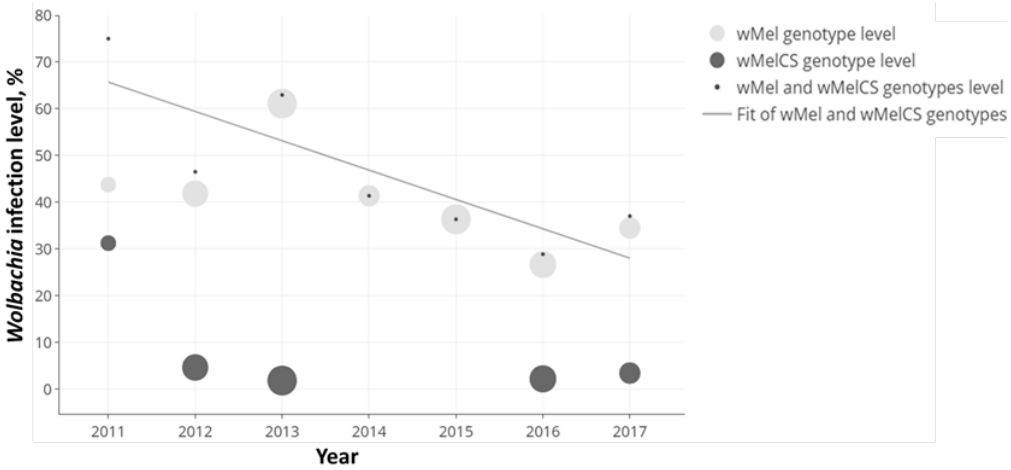

Fig. 2. Infection frequencies of natural population of *D. melanogaster* from Uman'
(Size of circles is proportional to log(N), where N = sample size)

Based on obtained data (table 1), we have performed general multiple linear model for average values of each climate parameters: *w*Mel/*w*MelCS ~ TA + DA + PA + TA × DA + DA × PA. Other models that have maximum, minimum and mixed variables of climate parameters are not shown because of the insignificance of each one (p-values varied from 0.06 to 0.7).

The model of *w*MelCS for summer season was significant ($F(5,1) = 429.4$, $p = 0.03662$, $R^2=0.9995$) (Model I). ANOVA for this model showed that PA ($F = 1673.711$, $df = 1$, $p = 0.016$) and TA × DA ($F = 207.582$, $df = 1$, $p = 0.044$) has significant influence on *w*MelCS frequencies. The same model for winter season showed insignificant result ($p = 0.5498$) as well as model of *w*Mel genotype with parameters of both summer ($p = 0.3169$) and winter ($p = 0.9989$) (Model II). The details of the models presented in the Table 2.

Table 1

The average values of climate factors and infection rates of two examined *Wolbachia* genotypes from Uman' for 7 years of study

Year	Sample size, n	*w*Mel, %	*w*MelCS, %	Temperature, °C		Dew point, °C		Precipitation, cm	
				S	W	S	W	S	W
2011[1]	16	44 (20 – 70)[3]	31(11 – 59)[2]	20	-4	14	-7	2	0.4
2012[1]	43	42 (27 – 58)[3]	5 (0.6 – 16)[2]	21	-6	13	-10	1	3.2
2013	54	61 (47 – 74)[3]	2 (0.05 – 10)[2]	19	-2	14	-4	1	0.8
2014[2]	29	41 (24 – 61)[3]	0	19	-3	13	-6	1	0.4
2015	55	36 (24 – 50)[3]	0	20	-1	13	-4	1	0.5
2016	45	27 (15 – 42)[3]	2 (0.06 – 12)[2]	20	-2	14	-4	1	1.05
2017	27	34 (17 – 54)[3]	3 (0.09 – 19)[2]	20	-4	13	-7	1	0.45

[1]from Serga et al. [20] and [2]Gora et al. [6]; [3]Clopper-Pearson's confidence interval; S – summer, W – winter, n – number of isofemale lines

Table 2

Outcome of multiple linear regression models based on relationship between *w*Mel/*w*MelCS yearly levels and climate variables

(Significant values are marked by bold font)

Model	Variables	Summer				Winter			
		Estimates	SE	t-value	p-value	Estimates	SE	t-value	p-value
I	Intercept	5073.525	364.665	13.91	**0.0457**	-148.2706	141.2795	-1.049	0.485
	TA	-240.660	18.301	-13.15	**0.0483**	43.5631	24.8664	1.752	0.330
	DA	-380.560	27.045	-14.07	**0.0452**	-47.1674	43.2375	-1.091	0.472
	PA	-333.543	31.434	-10.61	0.0598	98.7794	56.1602	1.759	0.329
	TA × DA	18.019	1.356	13.29	**0.0478**	0.6592	6.1077	0.108	0.932
	DA × PA	26.279	2.309	11.38	0.0558	13.3595	6.9676	1.917	0.306
II	Intercept	-11551.01	3065.34	-3.768	0.165	83.148	372.250	0.223	0.860
	TA	599.30	153.84	3.896	0.160	-18.484	65.519	-0.282	0.825
	DA	868.28	227.34	3.819	0.163	12.786	113.924	0.112	0.929
	PA	-465.38	264.23	-1.761	0.329	-46.270	147.974	-0.313	0.807
	TA × DA	-44.86	11.40	-3.935	0.158	-1.078	16.093	-0.067	0.957
	DA × PA	34.06	19.41	1.755	0.330	-6.014	18.359	-0.328	0.798

I, II – models of *w*MelCS and *w*Mel, respectively; SE – standard error

The Model I for summer indicates that average temperature and dew point variables have possible influence on *w*MelCS dynamics. For further validation of this model we developed the prediction model based on Model I and compared expected *w*MelCS levels with observed one. Based on estimates from table 2, we obtained the following equation: predicted level of *w*MelCS = - 240.660 × TA -

380.560 × DA - 333.543 × PA + 18.019 × TA × DA + 26.279 × DA × PA + 5073.525. The predicted ones *w*MelCS levels were plotted against observed ones those in this study (fig. 3).

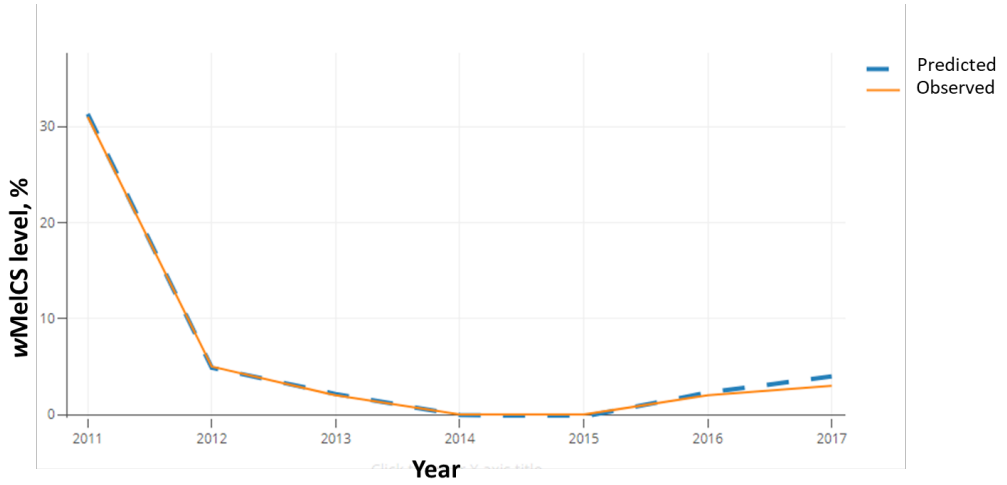

Fig. 3. Correlation of observed and predicted levels of wMelCS genotype for 2011–2017

Predicted and field data have significant correlation between each other (Pearson correlation coefficient: $r = 0.9997$, $p < 0.001$).

Discussion

Stable co-existence of two genotypes of *Wolbachia* is unique quality of *D. melanogaster* population in Uman' locality. Besides, it has recorded here since mid-20th century. We suggest that one of the causes of continuous *w*MelCS presence in this population can be that the infected flies might be re-established after winter in the local refugia such as a juice factory in this town. This hypothesis is also supported by the fact that Serga et al. [20] surveyed two other population from Uman' and they were infected only with *w*Mel genotype.

Our results indicate that the dynamics of *w*MelCS have been affected by climate factors during the years of monitoring. This can be attributed to the changes of climate in the northern hemisphere as well as in Ukraine. During 112 years the average temperature has increased on 0.8–1 °C in the region where Uman' is located. The more intensive increase of temperature was observed for the last ten years (on 0.3 °C) in Ukraine, indicating the warming process [13].

Our results indicate that the infection rate has propensity to decline over the last decade in *D. melanogaster* population in Uman'. Truit et al [24] have shown that infected flies prefer a lower range of temperature than uninfected ones. This behavioral adaptation is likely affecting the accuracy of the estimation of the link between temperature and infection frequencies. Thus, such a relationship has to be observed over the influence of narrow interval of temperature during the season.

Conclusions

Relying on previous experimental evidence and our observations we

speculate that *w*MelCS frequencies are affected by climate factors *D. melanogaster* population from Uman`. Nevertheless, the relationships between environmental factors and infection dynamics need to be investigated in the combination of both field and laboratory studies.

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ДИНАМІКА ЧАСТОТ ГЕНОТИПІВ *WOLBACHIA* В ПРИРОДНІЙ ПОПУЛЯЦІЇ *DROSOPHILA MELANOGASTER* З УМАНІ ПРИ ВПЛИВІ КЛІМАТИЧНИХ ФАКТОРІВ

Реферат

Мета. *Визначити можливий вплив кліматичних параметрів на частоту генотипів Wolbachia в популяції Drosophila melanogaster Умані протягом семи років.* ***Методи.*** *Для визначення рівня інфікованості Wolbachia в ізосамкових лініях, збір яких здійснювався впродовж літньо-осіннього періоду 2013 р. та 2015–2017 рр. з Умані, було проведено полімеразну ланцюгову реакцію (ПЛР) зі специфічними праймерами до генів 16S rRNA та wsp (Wolbachia surface protein) бактерії. Для визначення wMel та wMelCS генотипів Wolbachia було проведено ПЛР з маркерами наявності IS5 інсерції в локусі WD1310 та зміни кількості мінісателітних повторів у VNTR-141 локусі. Результати статусу інфекції мух за 2011–2012 рр. та 2014 р. були включені з наших попередніх робіт. Дані кліматичних факторів (середня температура, точка роси та кількість опадів за сезон) для зими та літа кожного року дослідження були отримані з погодних баз даних. Статистичний аналіз взаємодії генотипів Wolbachia та кліматичних факторів було проведено в R v.3.5.0, використовуючи множинний регресійний аналіз.* ***Результати.*** *Виявлено зниження частоти інфікованості Wolbachia та переважання wMel генотипу в природній популяції Drosophila melanogaster Умані протягом років моніторингу. Також було встановлено низькі частоти рідкісного в природних умовах wMelCS генотипу. Частота wMelCS була зумовлена сумісним впливом середньої температури та вологості влітку (p = 0.03662, R^2 = 0.9995).* ***Висновки.*** *Кліматична мінливість впливає на генотип wMelCS бактерії Wolbachia у природній популяції D. melanogaster з Умані.*

Ключові слова: Drosophila melanogaster, Wolbachia, wMelCS, дрозофіла, ендосимбіонт, кліматичні фактори, Умань, Україна.

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ДИНАМИКА ЧАСТОТ ГЕНОТИПОВ *WOLBACHIA* В ПРИРОДНОЙ ПОПУЛЯЦИИ *DROSOPHILA MELANOGASTER* ИЗ УМАНИ ПРИ ВОЗДЕЙСТВИЮ КЛИМАТИЧЕСКИХ ФАКТОРОВ

Реферат

Цель. *Определить возможное влияние климатических параметров на частоту генотипов Wolbachia в популяции Drosophila melanogaster Умани в течение семи лет.* ***Методы.*** *Для определения уровня инфицированности Wolbachia в изосамочных линиях, сбор которых осуществлялся в течение летне-осеннего периода 2013 г. та 2015–2017 гг. из Умани, была проведена полимеразная цепная реакция (ПЦР) со специфическими праймерами к генам 16S rRNA и wsp (Wolbachia surface protein) бактерии. Для определения wMel и wMelCS генотипов Wolbachia было проведено ПЦР с маркерами наличия IS5 инсерции в локусе WD1310 и изменения количества минисателлитных повторов в VNTR-141 локусе. Результаты статуса инфекции мух за 2011–2012 гг. и 2014 г. были включены из наших предыдущих работ. Данные климатических факторов (средняя температура, точка росы и количество осадков за сезон) для зимы и лета каждый год исследования были получены из погодных баз данных. Статистический анализ взаимодействия генотипов Wolbachia и климатических факторов было проведено в R v.3.5.0, используя множественный регрессионный анализ.* ***Результаты.*** *Выявлено снижение частоты инфицированности Wolbachia и доминирование wMel генотипа в природной популяции D. melanogaster Умани в течение семи лет мониторинга. Также было установлено низкие частоты редкого в естественных условиях wMelCS генотипа. Частота wMelCS была обусловлена совместным влиянием средней температуры и влажности летом (p = 0.03662, R^2 = 0.9995).* ***Выводы.*** *Климатическая изменчивость влияет на генотип wMelCS бактерии Wolbachia в природной популяции D. Melanogaster из Умани.*

Ключевые слова: Drosophila melanogaster, Wolbachia, wMelCS, дрозофила, ендосимбионт, климатические факторы, Умань, Украина.

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