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Short communication

DETECTION OF *WOLBACHIA* IN LARVAE OF *LOXOSTEGE STICTICALIS* (PYRALOIDEA: CRAMBIDAE) IN EUROPEAN AND ASIAN PARTS OF RUSSIA

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Beet webworm *Loxostege sticticalis* is a notorious pest widely distributed on the territory of Eurasia. Its outbreaks cause severe damage to crops in Russia and China. Here *Wolbachia* infection is reported for the first time in *L. sticticalis*. Larvae were sampled in Rostov, Saratov, Irkutsk Regions and Republic of Buryatia in 2005–2013. Primers targeting the *wsp* gene were used for the PCR screening of *Wolbachia*. Among 148 larvae, 35 were *Wolbachia* positive. *Wolbachia* prevalence rate ranged from 21 to 40% in the Asian and from 0 to 47% in the European part of Russia. The combined sample subsets were compared for European versus Asian part of Russia and 2005–2009 versus 2010–2013 timeframes. The prevalence rates of *Wolbachia* were not significantly different between two parts of Russia, but the endosymbiont presence (estimated for the total dataset) increased with time within the observation period.

Keywords: beet webworm, endosymbiont, prevalence rate, PCR

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Introduction

Beet webworm *Loxostege sticticalis* L. is a notorious pest of numerous crops in European and Asian parts of Russia (Frolov et al., 2008), as well as in Northern China (Chen Xiao et al., 2008). Screening insect populations for naturally occurring parasites and pathogens is crucial for understanding the dynamics of pest populations. In particular, obligate intracellular parasites, such as Microsporidia, play an essential role in *L. sticticalis* density dynamics (Frolov et al., 2008). So far, other intracellular symbionts in populations of *L. sticticalis* have not been reported.

Bacteria of the *Wolbachia* genus are widespread endosymbionts of arthropods (Jeyprakash, Hoy, 2000). In certain species of Lepidoptera, *Wolbachia* may contribute to population biology of the hosts (Salunkhe et al., 2014), as it regulates reproductive processes (including sex determination) and influences host vitality and fertility in direct or indirect ways (Kageyama et al., 2002; Kageyama, Traut, 2004). The knowledge of *Wolbachia* distribution in insect populations is therefore of great interest, being important for a better understanding of the mechanisms underlying regulation of pest density dynamics (Sumi et al., 2017). Here, we report the first results of screening *Wolbachia* infection in *L. sticticalis* populations.

Materials and Methods

Beet webworm larvae were collected on crops and weeds in European and Asian parts of Russia (Fig. 1A). Insects were fixed with ethanol and stored at –20 °C. Total DNA was extracted using a simplified protocol of Sambrook et al. (1989) without addition of phenol. For quality control of DNA samples, the primers LepF1/LepR1 (Hebert et al., 2004) specific for the barcoding region of mitochondrial cytochrome oxidase subunit I (*COI*) were used. DNA samples producing a specific signal with *COI*-targeted primers were selected for further analysis. The *Wolbachia* infection was detected by amplification with primer set wsp81F/wsp691 (Zhou et al., 1998), specific to the locus of *Wolbachia* surface protein (*wsp*). We used DreamTaq Green PCR Master Mix (Thermo Fisher Scientific) with the following cycling conditions: initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, elongation at 72 °C for 1 min, and final elongation step of 72 °C for 5 min. The amplicons were visualized using electrophoresis in 1% agarose gels with GeneRuler Ladder Mix molecular weight marker, 75-20000 bp (Thermo Fisher Scientific). The 95% confidence intervals were estimated using the Clopper-Pearson method (Clopper, Pearson, 1934) which is routinely used when *Wolbachia* prevalence rates in small samples are examined (Yudina et al., 2016, Bykov et al., 2019). Estimates of the data reliability were obtained using the exact Fisher's test (Fisher, 1922), Pearson's chi-square criterion and chi-square criterion with Yates correction (Yates, 1934).

Results and Discussion

PCR with primers specific for *wsp* gene fragment of *Wolbachia* has yielded amplicons with the expected size of ~600 bp (Fig. 1B) in 35 out of 148 analyzed samples. In a single sample set collected in 2005 from Rostov Region, *Wolbachia* infection has been detected in 2 out of 24 larvae, corresponding to the prevalence rate of 8.3%. Thirty larvae collected in Saratov Region have been found as *Wolbachia*-free in 2006, but nearly half have been *Wolbachia*-positive in 2013. In the Asian part of Russia, *Wolbachia* prevalence has ranged from 16.7 to 40% (Table 1). The quotes of infected insects have been significantly different in a pairwise comparison of populations in 50% of cases. In particular, population Salsk 2005 differs from Saratov 2013 and Irkutsk 2010, while Saratov 2006 differs from Saratov 2013, Irkutsk 2010 and Kabansk 2009 (Table 2).

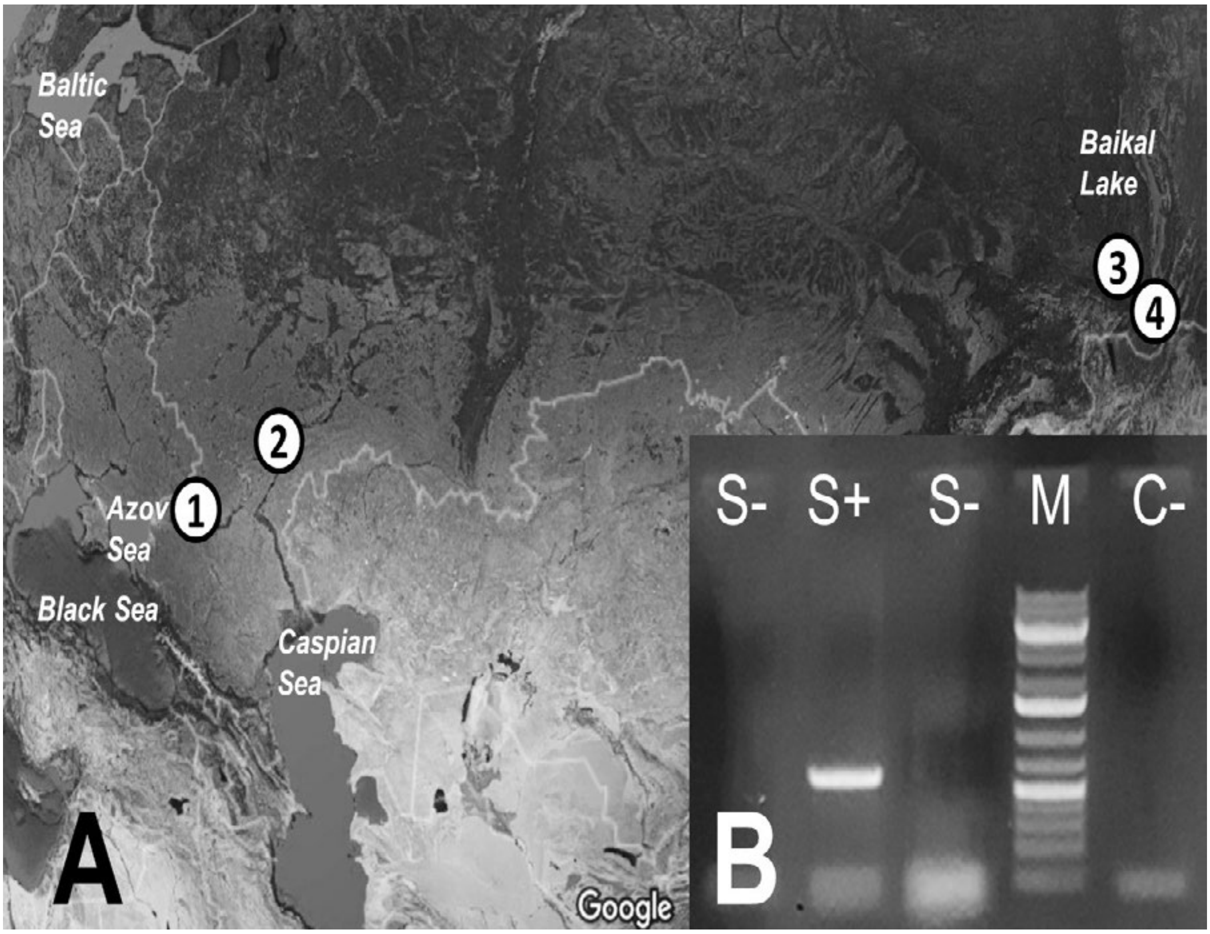

Figure 1. Detection of *Wolbachia* in *Loxostege sticticalis*. A: Sampling sites of *L. sticticalis* larvae in Salsk, Rostov region (1), Saratov, Saratov Region (2), Irkutsk, Irkutsk Region (3) and Kabansk, Buryatia (4). B: Electrophoretic profile of PCR samples negative (S-) and positive (S+) for *Wolbachia,* GeneRuler Ladder Mix molecular weight marker, 75-20000 bp (M) and negative control (C-)

Table 1. Prevalence rates of *Wolbachia* in *Loxostege sticticalis* larvae sampled across Russia

#	Sampling site, year, collector	Coordinates	Number of analyzed samples (N)	*Wolbachia* prevalence rates		
				Number of positive samples, n	Prevalence, % (n/N)	95 % confidence interval, %
1	Salsk, Rostov region, 2005, Malysh J.M., Tokarev Y.S.	46°30’N 41°19’E	24	2	8.3	1.0–27.0
2	Saratov, Saratov Region, 2006, Silaev A.I.	51°27’N 46°12’E	30	0	0.0	0.0–11.6
3	Saratov, Saratov Region, 2013, Silaev A.I.	51°27’N 46°12’E	30	14	46.7	28.3–65.7
4	Irkutsk, Irkutsk Region, 2010, Belyakova N.A.	52°16’N 104°19’E	30	12	40.0	22.7–59.4
5	Kabansk, Buryatia, 2009, Akhanaev Y.B.	52°09’N 106°36’E	34	7	20.6	8.7–37.9
Total			148	35	23.6	-

Table 2. Statistical significance of differences of *Wolbachia* prevalence between *Loxostege sticticalis* larval populations according to exact Fisher’s test

Local population (place and year)	Pairwise p-values using exact Fisher’s test				
	Salsk 2005	Saratov 2006	Saratov 2013	Irkutsk 2010	Kabansk 2009
Salsk 2005	=	0.1929	**0.0136**	**0.0074**	0.1394
Saratov 2006		=	**0.0002**	**0.0001**	**0.0087**
Saratov 2013			=	0.2020	0.0816
Irkutsk 2010				=	0.0533
Kabansk 2009					=

To further test possible differences in *Wolbachia* prevalence rates over time and place, we have compared the combined sample subsets of European versus Asian part of Russia and 2005–2009 versus 2010–2013 timeframes. In European part of Russia (Salsk + Saratov), the average *Wolbachia* prevalence rate have been 19.0% (N=84), while in Asian part (Irkutsk + Kabansk) this index has reached 29.7% (N=64). According to Pearson's chi-square criterion (χ^2=2.271), the prevalence rates of *Wolbachia* does not depend on the geographical origin of the sampled populations, which indirectly confirms the conclusion that the beet webworm populations belong to a single metapopulation (Jiang et at., 2010). Meanwhile, in 2005–2009 (number of positive samples per 30 samples <10) and 2010–2013 (number of positive samples per 30 samples >10), the average *Wolbachia* prevalence rates were 10.7% (N=88) and 43.3% (N=60), respectively. According to chi-square criterion with Yates correction for continuity (χ^2=19.819), the *Wolbachia* prevalence rate does not depend on the sampling timeframe (p=0.01). Similar trend have been observed when the timeframe of 2005–2006 (3.7%) have been tested against 2009–2013 (35.1%). These findings clearly indicate that the endosymbiont prevalence rate have increased with time within the observation period.

Prevalence rates of *Wolbachia* in insect hosts may significantly vary over time and space. For example, in pyraloid moths of the genus *Ostrinia, Wolbachia* has been found in all examined populations in European part of Russia, and the prevalence rates have depended on the species and the forage plant (Tokarev et al., 2018). Long-distance migrations are likely to provide symbiont exchange between local populations of the beet webworm, but various factors may affect the temporal dynamics of *Wolbachia* infection, revealed in the present study.

According to the Russian Agricultural Center (https://rosselhoscenter.com/), low density of beet webworm was reported in the Russian Federation in 2005 and 2006. From 2008 to 2014, the period of relatively high density was observed in Russia, with maximum in 2009, when the pest outbreaks occurred throughout the entire pest area from the Southern and Central Federal Districts to the Far Eastern Federal District. We have noticed a trend that during the period of low pest abundance, infection rate of *Wolbachia* was at its minimum, while during the period of high abundance, it was increasing over time. A long depression period of the pest, observed from 2015 to 2018, has been followed by an increase in the number of the beet webworm in Siberia. In the coming years, we expect to collect more data and to verify the relationship between the pest number and the frequency of *Wolbachia* infection.

The *Wolbachia* infection has been found for the first time in populations of *L. sticticalis* in the present study. Although the sampling sites are not numerous, it is obvious that the bacterium is present in the majority of local samplings. The beet webworm tends to form a single metapopulation on the territory of Eurasia due to its high migratory activity. In a given locality, the endosymbiotic bacterium may change its state over time from absence (presence at undetectable levels) to presence in a half of the insect population, as shown for the samplings from Saratov. The examined dataset does not allow to determine whether the fluctuations of *Wolbachia* prevalence rates is adaptive or stochastic. Further studies are necessary to elucidate the genetic diversity of *Wolbachia* and its biological role in populations of the beet webworm.

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Краткое сообщение

ОБНАРУЖЕНИЕ *WOLBACHIA* В ГУСЕНИЦАХ *LOXOSTEGE STICTICALIS* (PYRALOIDEA: CRAMBIDAE) В ЕВРОПЕЙСКОЙ И АЗИАТСКОЙ ЧАСТЯХ РОССИИ

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Луговой мотылёк *Loxostege sticticalis* – общеизвестный вредитель, широко распространённый на территории Евразии. Его вспышки вызывают серьёзные повреждения сельскохозяйственных культур в России и Китае. Здесь мы впервые сообщаем о заражённости *L. sticticalis* вольбахией. Гусеницы были собраны в Ростовской, Саратовской, Иркутской областях и Республике Бурятия в 2005–2013. Для скрининга использовали ПЦР со специфичными праймерами, нацеленными на ген вольбахии *wsp.* Среди 148 гусениц было обнаружено 35 особей, давших положительный сигнал на *Wolbachia*. Показатель распространённости вольбахии варьировал от 21 до 40% в азиатской и от 0 до 47% в европейской частях России. Объединённые выборки сравнивались по месту сбора насекомых (европейская и азиатская части России) и по годам сборов (2005–2009 и 2010–2013). Показатели распространённости *Wolbachia* достоверно не различались между двумя частями России, при этом присутствие эндосимбионта (в отношении общей выборки) увеличивалось со временем в течение периода наблюдения.

Ключевые слова: луговой мотылёк, эндосимбионт, уровень распространённости, ПЦР

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