ISSN 1062-3590, Biology Bulletin, 2023, Vol. 50, No. 3, pp. 338–347. © The Author(s), 2023. This article is an open access publication, corrected publication 2023.
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MICROBIOLOGY

Ectoparasitic Mites: Vectors of Bacterial Symbionts among Insects

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Received May 11, 2022; revised October 5, 2022; accepted October 5, 2022

Abstract—Inherited endosymbiotic bacteria from the genera *Rickettsia, Wolbachia,* and *Spiroplasma* cause the death of male offspring in ladybirds (Coleoptera, Coccinellidae). As a rule, bacteria are transmitted through the cytoplasm of the mother's egg to offspring, vertically. In addition to vertical transfer, there is increasing evidence of horizontal transfer of symbionts between unrelated insect taxa. Insect parasites such as mites can be potential vectors of endosymbiotic bacteria. The parasitic mite *Coccipolipus hippodamiae* (McDaniel & Morrill, 1969) (Acarina: Podapolipidae) occurs in natural populations of Coccinellidae. In this work, the ability of *C. hippodamiae* to become infected with *Wolbachia* and *Spiroplasma* from hosts and to spread bacteria among coccinellid beetles was proven for the first time.

Keywords: intracellular symbiotic bacteria, horizontal transfer, insects

DOI: 10.1134/S1062359023700231

INTRODUCTION

Inherited endosymbiotic bacteria are ubiquitous in natural populations of invertebrates. Intracellular bacterial symbionts of insects are characterized by a wide range of interactions with the host, which make it possible to influence the ecology, evolution, and reproductive biology of the latter. An exceptional feature is the ability to cause a number of reproductive anomalies in their hosts (cytoplasmic incompatibility, male-killing, feminization, or parthenogenesis), which increase the proportion of infected females in the population and, accordingly, the efficiency of their vertical transmission and spread in the population (Werren et al., 1995).

Ladybirds (Coleoptera, Coccinellidae) in Russia have three inherited symbionts from the genera *Rickettsia*, *Wolbachia,* and *Spiroplasma,* causing the death of male offspring, i.e., male-killing (Shaikevich and Zakharov, 2015; Goryacheva et al., 2015, 2018; Shaikevich et al., 2019). The frequency of occurrence and the geographical distribution of symbionts are not the same in various species. For *Adalia decempunctata* a typical infection is with *Rickettsia* (Shaikevich et al., 2019). In *Harmonia axyridis, Wolbachia*, *Rickettsia,* and *Spiroplasma* were discovered (Goryacheva et al., 2015, 2017, 2018; Li et al., 2021). In the population of *Adalia bipunctata* in Russia, the geographical distribution of symbiotic bacteria was observed: in St. Petersburg, *Rickettsia* and *Spiroplasma* were found in 1999 (Schulenburg et al., 2002) and exclusively *Spiroplasma* was found in 2009 (Zakharov and Shaikevich, 2011), while in Karelia and Buryatia, only *Rickettsia* was discovered (Shaikevich et al., 2012). In the *A. bipunctata* ladybirds, in one population in Moscow in 2019–2020, infection with at least three strains of *Wolbachia* was detected, *w*Abi-1, *w*Abi-2, and *w*Abi-3 (Shaikevich et al., 2021), two of which were not found in the same population in 1999 (Schulenburg et al., 2002). Long-term observations show that the composition of symbionts in ladybird populations can change over time due to the loss of some bacteria and the acquisition of others.

Intracellular symbiotic bacteria infect the germline cells of the host and are transmitted through the cytoplasm of the egg, i.e., transovarially from mother to offspring or vertically. In addition to vertical transfer, there is increasing evidence of horizontal transfer of symbionts between unrelated insect taxa. Cases of infection of insects with bacteria as a result of direct and indirect contacts (as a result of living in the same environment, contact between predator and prey, or through a common food source) are known (cited by Pietri et al., 2016). The possibility of horizontal transmission in nature is also indicated by phylogenetic data (O'Neill et al., 1992; Baldo et al., 2008; Gerth et al., 2013; Ahmed et al., 2016; Ilinsky and Kosterin, 2017; etc.).

Insect parasites such as mites can be potential vectors of endosymbiotic bacteria. It has been shown that ectoparasitic mites *Macrocheles subbadius* after feeding on the hemolymph *Drosophila nebulosa,* infected with *Spiroplasma,* are capable of transmitting the infection to *Drosophila willistoni* (Jaenike et al., 2007). *Drosophila hydei* captured in nature were found with *Macrocheles* sp. mites infected by *Spiroplasma* identical to the host symbiont (Osaka et al., 2013). A com-

pletely different mechanism is found at the heart of the transfer of *Wolbachia* between laboratory populations of *Drosophila* through *Tyrophagus putrescentiae*: these mites eat *Drosophila* corpses, including those infected with *Wolbachia*, and *Drosophila* larvae eat the mites and thus become infected with *Wolbachia* (Brown and Lloyd, 2015).

The parasitic ladybird mite *Coccipolipus hippodamiae* (McDaniel & Morrill, 1969) (Acarina: Podapolipidae) is found in natural populations of Coccinellidae (Coleoptera) in which it can reach high numbers (Webberley et al., 2004). *C. hippodamiae* was found in different species of coccinellids: *A. bipunctata*, *A. decempunctata*, *Oenopia conglobata*, *Calvia quatuordecimguttata*, *Coccinella magnifica*, *Harmonia quadripunctata*, *H. axyridis*, *Hippodamia convergens*, *Exochomus fulvimanus*, and *Exochomus concavus* (Knell and Webberley, 2004; Webberley et al., 2004; Rhule et al., 2010; Ceryngier et al., 2012). Some species of ladybirds do not appear to be infested with *C. hippodamiae* mites: *Exochomus quadripustulatus*, *Coccinula quatuordecimpustulata*, *Propylea quatuordecimpunctata*, and *Coccinella septempunctata* (Webberley et al., 2004). *C. septempunctata* is parasitized by another mite *Coccipolipus macfarlanei* (Eidelberg, 1994; Zakharov and Eidelberg, 1997; Knell and Webberley, 2004). In Europe, the highest mite infestation by *C. hippodamiae* (up to 69.5%) was observed on *A. bipunctata*, making it possible to consider this species of ladybirds as its main host (Webberley et al., 2004). However, the areal of *C. hippodamiae* does not match the areal of *A. bipunctata* (Zakharov and Eidelberg, 1997; Webberley et al., 2006).

C. hippodamiae is an ectoparasite that lives on the underside of the elytra of coccinellids and is transmitted mainly during copulation, as well as in dense clusters of beetles preparing for diapause (Webberley and Hurst, 2002). Adult female mites lead a motionless life: they attach to the elytra, absorb the host's hemolymph, and lay eggs, from which mobile translucent whitish larvae emerge. During beetle copulation, mite larvae migrate under the elytra of a new host, where young females begin to feed on the hemolymph and undergo metamorphosis, turning into adults. After that, adult females stop moving, eventually increase in size, become yellow–orange in color, and begin to lay eggs. Fertilization of female mites occurs at the nymph stage (Ceryngier et al., 2012). Spreading of *C. hippodamiae* depends, for the most part, on two factors: on the severity of host promiscuity, which contributes to the transmission of the parasite between individuals, and on the duration of coexistence of different generations of hosts during periods of continuous reproduction, because it provides transmission of *C. hippodamiae* between generations of beetles (Webberley et al., 2004). The spread of mites between coccinellids of different species has been found in nature in places where at least one species of coccinellids has been infested with *C. hippodamiae* (Webberley et al., 2004). In laboratory experiments *C. hippodamiae* successfully reproduced on a previously uninfected host after sexual contact of individuals of heterospecific pairs (Rhule et al., 2010). Mites are able to adapt to different species and genera of ladybirds, and in experiments there was no significant difference in the time required for successful reproduction of mites on *H. axyridis* and on *A. bipunctata* (Rhule et al., 2010).

The purpose of this work was to investigate whether *C. hippodamiae* carry out horizontal transfer of symbionts between coccinellids. We assumed that the mite *C. hippodamiae* can acquire symbionts by absorbing the hemolymph of an infected host and transmit the bacterium to offspring. Young nymphs of such mites crawl under the elytra of new hosts and, starting to feed on the hemolymph, can infect previously uninfected beetles with the bacterium.

MATERIALS AND METHODS

Imago ladybirds (*A. bipunctata* and *H. axyridis*) were collected in 2019–2021 by visual examination of shrubs and trees (during the warm season) or walls of buildings (in autumn), on which beetles preparing for diapause can be found. Female *A. decempunctata* ladybirds, used for this experiment, were bred from pupae collected in nature earlier (Romanov and Matveikina, 2021). The collected beetles were given individual names, which indicated the place of collection (M, the city of Moscow) and the serial number of the collected beetle. In the names of female ladybirds related to the species *A. decempunctata* and *H. axyridis*, their species affiliation was indicated by the lowercase Latin letters "d" and "a" after the serial number (for example, M84d and M150a, respectively). Beetles collected in nature and used to obtain laboratory lines were marked with a capital Latin letter P (from the word "parenta," parents). Their descendants were marked with a capital Latin letter F (from the word "filii," children) indicating the generation number.

Adult female *C. hippodamiae* mites are located on the inner side of the elytra of ladybirds (Fig. 1a), so collection of them from a live beetle is difficult. Nymphs and, possibly, adult male mites are mobile; we noted these forms on the surface of elytra of infected beetles (Fig. 1b). Therefore, we assumed that mite nymphs would crawl from mite-infested ladybirds to other beetles not only during copulation (the most common natural type of mite transmission), but also when kept together. This assumption was confirmed, since in several Petri dishes, where only female ladybirds were kept, infestation of beetles placed there with mites was noted. Infestation with mites was diagnosed visually using an MBS-10 binocular microscope by the presence of mobile forms of mites on the elytra of ladybirds and the type of the eggs laid by female coccinellids (in females ladybirds infected with mites, the eggs shrivel a few hours after laying; the effect begins to appear approximately three

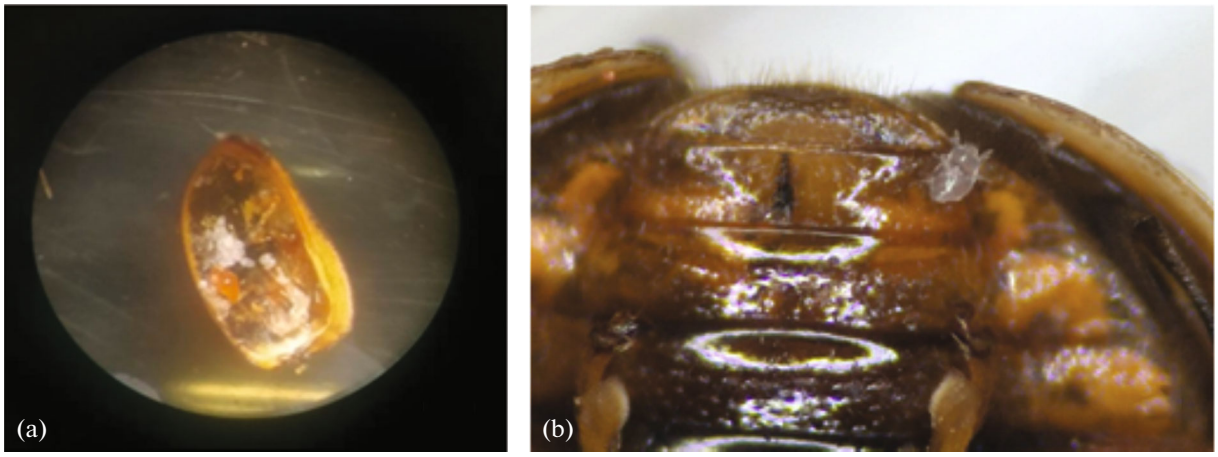

Fig. 1. (a) Adult female *Coccipolipus hippodamiae* mite (yellow) and her eggs (white) in the elytra of an *Adalia bipunctata* ladybird; (b) mobile nymph (larva) of the mite.

weeks after infestation). In a number of cases, to check the success of infestation with mites, ladybirds were euthanized with diethyl ether, the elytras were carefully folded under a microscope, and the presence of mites was observed. The number of beetles contained in a Petri dish depended on its diameter: in Petri dishes with a diameter of 4 cm, there were 3–4 beetles; in Petri dishes with a diameter of 8 cm, there were 6–8 beetles. In this way, we simulated the ecological situation in nature, where ladybirds become infected with mites during copulation or in dense clusters of wintering beetles. After the death of the beetle, individual mites were removed from the elytra and the DNA was isolated from the beetle and from the mites (individually and from groups of 2–8 mites) to search for symbionts in the host and parasite by PCR.

Total DNA isolation from individual mites and their coccinellid hosts was performed using the DNA Prep kit (Isogen, Moscow). The amplification reaction with each DNA preparation was carried out in a volume of 25 μL using the universal Encyclo Plus PCR kit (Evrogen, Moscow) in accordance with the manufacturer's protocol. All reactions were conducted on a MiniAmp Plus amplifier (Applied Biosystems, United States). To amplify the *cox1* gene, universal primers were used: LCO1490 and HCO2198 (Folmer et al., 1994); the amplification conditions were initial denaturation, 4 min 30 s at 94°C; then five cycles: denaturation for 30 s at 94°C, annealing for 20 s at 45°C, and synthesis for 1 min at 72°C; then 35 cycles: denaturation for 30 s at 94°C, annealing for 20 s at 55°C, and synthesis for 1 min at 72°C. PCR was completed by final synthesis for 5 min at 72°C. Amplification of the *cox1* specific gene fragment of the ladybirds was carried out with primers C1-jF 5'-GCTGGAATTTCATCAATTTTAGG-3' and C1-nR 5'-GGAAATCAATGAATAAATCCTGCT-3'. The PCR conditions were primary denaturation, 5 min at 94°C; 38 cycles using Encyclo polymerase: denaturation at 94°C for 30 s, annealing at 59°C for 30 s, and synthesis at 72°C for 60 s; and final synthesis at 72°C, 5 min.

Mite and ladybird infestation by *Wolbachia* were checked by PCR according to the MLST analysis method (http://pubmlst.org/wolbachia). To test for bacterial infestation of *Spiroplasma*, the primers Sp_ApDnaA_F1 5'-ATTCTTCAGTAAAAATGCTTGGA-3' and Sp_ApDnaA_R1 5'-ACACATTTACTTCATGCTATTGA-3' were used; for *Rickettsia,* RicF141 5'-TCGGTTCTCTTTCGGCATTTTA-3' and RicR548 5'-GCATATTTATCACCGCTTCATT-3'. The amplification conditions were the initial denaturation, 4 min 30 s at 94°C; then 35 cycles: denaturation for 25 s at 94°C, annealing for 20 s at 58°C, and synthesis for 35 s at 72°C. PCR was completed by final synthesis for 5 min at 72°C. The PCR results were analyzed by electrophoresis in 1.5% agarose gel. PCR-amplified products of the *cox1* fragments of mtDNA and loci of *Wolbachia* and *Spiroplasma* were sequenced.

Chromatograms of nucleotide sequences were analyzed using the DNASTAR Lasergene 6 software package (https://www.dnastar.com/software/lasergene/seqman). To identify insect species by comparing the obtained sequences with those already known, we used the international databases Barcode of Life Database (BOLD) and GenBank. The loci of *Wolbachia* were compared in the database http://pubmlst.org/wolbachia and GenBank. Newly obtained *dna*A gene sequences of *Spiroplasma* from *A. bipunctata* and *C. hippodamiae* were registered in GenBank under numbers ON382044 and ON382045, respectively. The phylogenetic dendrogram was built using the MEGA V 6 program using the Maximum Likelihood method, the Tamura-Nei model, and bootstrap support of 1000 replicas (Tamura et al., 2013).

Table 1. Collections of mites parasitizing *A. bipunctata*, in Moscow in 2019–2021

Collection time (month, year)	Gathering place	Laybirds collected		Mite infestation, %
		total	with mites	
October 2019	55°42′37″ N, 37°34′37″ E	35	12	34.3
June 2020	55°41′37″ N, 37°34′14″ E	21	10	47.6
June 2020	55°42′54″ N, 37°36′45″ E	42	32	76.2
May 2021	55°41′38″ N, 37°34′05″ E	44	5	11.4
May 2021	55°41′28″ N, 37°51′01″ E	49	5	10.2
Total		191	64	33.5

RESEARCH RESULTS

Natural Infestation of Female Ladybirds with C. hippodamiae mites and the Effect of Mites on Host Fertility

The collections of imagoes of *A. bipunctata* are presented in Table 1. Infection of populations of *A. bipunctata* by mites depends on the time and location, varying from 10.2 to 76.2% (Table 1). Collection of 112 imagoes of *H. axyridis* were produced only in August 2020 in Moscow (55°41′19″ N, 37°51′32″ E), none of them were infected with mites.

To study the effect of mites on the hatching of larvae of *A. bipunctata* from eggs, a comparison was made between two females infested with mites at about the same time (Table 2). The first M7(P)♀ is not infected with the symbiotic bacterium, and the second M14(P)♀ is infected with *Wolbachia*. In the offspring of the latter, half of the eggs hatched during the first week, which corresponds to the manifestation of male-killing caused by *Wolbachia*. In the offspring of the female not infected with the symbiont, the mites had no effect on the hatchability of the larvae and the appearance of the eggs chorion for the first nine days. On approximately the 11th–12th day of infection, there was a sharp increase in the number of underdeveloped eggs in both females, after which the female ladybirds became completely sterile. A week after the manifestation of sterility in females, the eggs they lay began to shrivel.

Infection of Mites (Adult Females) and Ladybirds with Bacterial Symbionts in Collections from Nature

We isolated DNA from *C. hippodamiae* female mites (Fig. 1a), taken from under the elytra of 12 *A. bipunctata* ladybirds and from ladybirds themselves, which were collected in nature in 2019 (Table 1). *Wolbachia* was detected by PCR with primers for the *ftsZ* bacterial gene in a pair of *A. bipunctata* and *C. hippodamiae* (sample M3). Later, six genes of *Wolbachia* from this mite and this ladybird were sequenced; the sequences of all genes (*gat*B MZ056866, *cox*A MZ056869, *hcp*A MZ056871, *fbp*A MZ056874, *ftsZ*-95, and *wsp*-392) are identical in mite and ladybird and correspond to the strain *w*Abi-1 (Fig. 2). In 2020 *C. hippodamiae* infected with symbionts were not found. In 2021 two females of *C. hippodamiae* were infected with *Wolbachia*; in the case of *A. bipunctata* M109, both the mite and the ladybird were infected, and in the case of *A. bipunctata* M90, only the mite. Seven lines were established for *C. hippodamiae*: two lines of mites infected with *Wolbachia*, and five lines not infected with symbiotic bacteria (Table 3). *Spiroplasma* or *Rickettsia* were not detected in *C. hippodamiae* from nature.

Among the beetles from the collections of 2021, in the offspring of one individual of *A. bipunctata* M98 *Spiroplasma* was discovered, and a laboratory line of ladybirds infected with *Spiroplasma* was established. In addition, the line of *A. bipunctata* M88 infected with *Wolbachia* is maintained in the laboratory from that found in nature. Symbionts were stably persisted in ladybird generations in the laboratory in 2021–2022, and ladybirds of the 3rd generation were infected in the lines M88 (with *Wolbachia*) and M98 (with *Spiroplasma*). Infection with symbionts was checked using PCR with primers to the *fts*Z and *cox*A genes of *Wolbachia* and the *dna*A gene of *Spiroplasma*.

In total, for experiments in 2021, eight lines of ladybirds were established and maintained: one line of *A. decempunctata* (M84d), one line of *H. axyridis* (M150a), six lines of *A. bipunctata*—M88 (source of *Wolbachia*), M98 (source of *Spiroplasma*) and M19, M26, M69, and M116 (free from bacteria). The experiments also used 18 beetles collected from nature, which were not bred in a line (Appendix 1).

To study the ability of *C. hippodamiae* mites to infect different types of ladybirds, the following experiment was conducted: along with *A. bipunctata* ladybirds that were infected with mites, uninfected *A. decempunctata* and *H. axyridis* ladybirds were placed. To study the possibility of *C. hippodamiae* mite infestation bythe bacterium *Wolbachia* or *Spiroplasma* in laboratory conditions, we conducted the following experiment: in Petri dishes with *A. bipunctata* ladybirds infected with a bacterial symbiont, but without mites, *A. bipunctata* adults were placed that were infected with mites. To study the ability of *C. hippoda-*

Table 2. Influence of mites on the hatchability of ladybird larvae from eggs

Line of ladybirds	Date of counting	Number of eggs		Number of larvae
		laid	undeveloped	
M7(P)♀W– mite infestation was June 6, 2020	June 10	39	2	37
	June 12	9	4	5
	June 13	34	1	33
	June 14	26	2	24
	June 16	3	0	3
	June 17	5	1	4
	June 18	29	20	9
	June 20	19	19	0
	June 24	21	21*	0
M14(P)♀W+ mite infestation was June 7, 2020	June 12	9	4	5
	June 13	30	13	17
	June 14	21	16	5
	June 16	4	2	2
	June 17	16	9	7
	June 18	23	20	3
	June 19	23	23	0
	June 20	19	19	0
	June 21	9	9	0
	June 24	3	3*	0

An asterisk (*) indicates eggs that began to shrivel a few hours after being laid.

miae mites to spread bacterial symbionts among ladybirds, the following experiment was performed: *A. bipunctata* ladybirds infected with symbiont-infected mites were joined by uninfected *A. bipunctata*, *A. decempunctata,* and *H. axyridis* ladybirds.

Experimental Proof of Infection of Mites with Symbionts from Ladybirds

To prove *C. hippodamiae* (Ch) infestation with bacteria directly from their ladybird hosts, *A. bipunctata* from (1) the M88W+ line (without mites, but the source of *Wolbachia*) and (2) the M98S+ line (no mites but a *Spiroplasma* source) were placed in Petri dishes with adult *A. bipunctata* infected with *C. hippodamiae* (Ch+) mites. Into the same dishes, *A. decempunctata* and *H. axyridis* ladybirds without mites (Ch–) were placed. Infection of ladybirds with mites was diagnosed by the presence of mobile forms of *C. hippodamiae* on the surface of the host elytra; all species of coccinellids were infected with *C. hippodamiae* mites in a few days. Infection with the symbiont was checked by PCR after the death of the ladybird. To check the contamination of mite DNA samples during DNA isolation, we conducted PCR with primers C1-jF and C1-nR, specific to the DNA of ladybirds alone. In the case of positive signals from mite DNA, such a sample was excluded from the analysis. As a result, we received evidence that mites become infected with *Wolbachia* and *Spiroplasma* (Table 3). After gene sequence analysis of *cox*A and *ftsZ* of the *Wolbachia* bacteria, it was shown that Ch52 W+ and the donor M88 W+ are infected with the strain *w*Abi-1 (Fig. 2). Identical *dna*A gene sequences of *Spiroplasma* were received for Ch59 S+ and the M98S+ donor (Fig. 2).

Mites Ch52 W+ and Ch59 S+ newly infected with symbionts retained symbionts when they infected other ladybirds (M69, M151a and M47, M150a, M162a, respectively) through mobile nymphs, which proves the heritability of acquired infections of *Wolbachia* and *Spiroplasma* in mites. Ladybirds M69 and M151a were not infected with the symbiont from the Ch52 W+ mite, nor were M47, M150a and M162a from the Ch59 S+. In the mite line Ch59, *Spiroplasma* was persisted for at least three generations of mites, and in the line Ch109, *Wolbachia* was transmitted over four generations of mites (Appendix 1). The number of generations of mites was determined by the lifetime of beetles infected with mites, comparing it with the

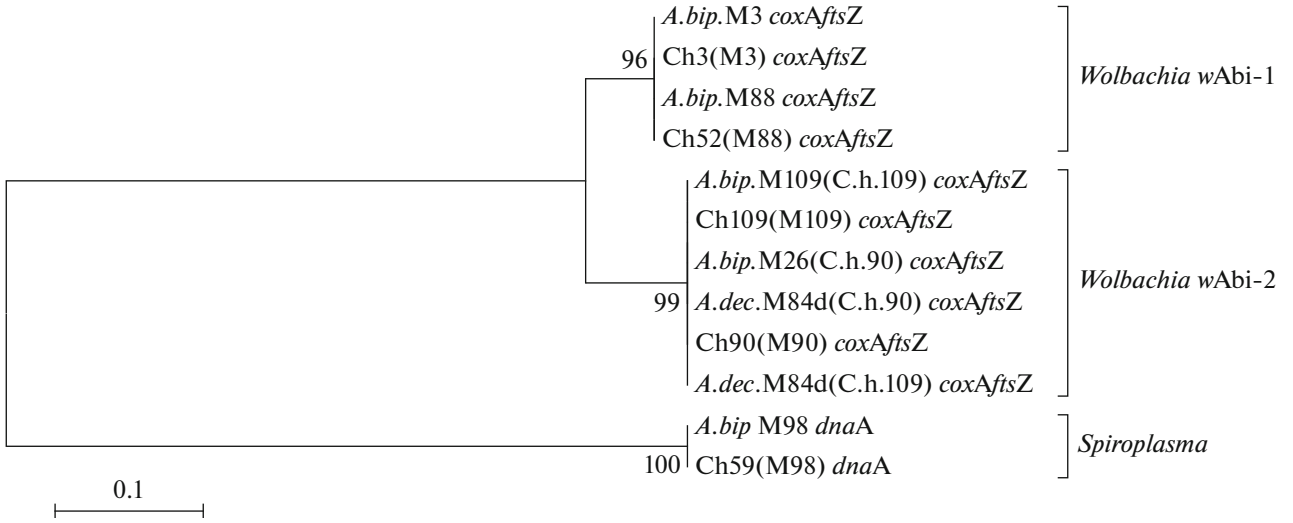

Fig. 2. Similarity dendrogram based on comparison of concatenated *Wolbachia cox*A-*fts*Z gene sequence and gene *dna*A of *Spiroplasma*. The hosts of symbionts are indicated—ladybirds or mites, and the strains of *Wolbachia* (on the right). When designating mites, the ladybirds from which they were taken are indicated in parentheses (e.g., Ch52 was taken from the ladybird of line (M88).

dates of introducing new ladybirds. Since adult female mites lead a stationary lifestyle, only their descendants can move to a new beetle.

Experimental Proof of Infection of Female Ladybirds through Mites

To test the ability of mites to transmit symbiotic bacteria, *A. bipunctata* ladybirds without symbionts or mites were placed into dishes with (3) *A. bipunctata* M109W+ Ch109W+ (infected simultaneously with mites Ch109 and *Wolbachia*) and (4) M90W– Ch90W+. The results proving the ability of coccinellids to become infected with *Wolbachia* through mites are presented in Table 3. To prove the absence of traces of beetle DNA in mite DNA samples, we conducted PCR with mite DNA and common primers LCO and HCO followed by sequencing. Chromatograms did not

Table 3. Experiment on the transfer of symbionts through mite bites

Symbiont recipient	Symbiont donor from nature			
	ladybirds		mites (host ladybird)	
	M88 W+ (Ch–)	M98 S+ (Ch–)	Ch109 W+ (M109 W+)	Ch90 W+ (M90 W–)
Mites (ladybird host)				
Ch52 W– (M52 W–)	W+			
Ch68 W– (M68 W–)	W–			
Ch59 S– (M59 S–)		S+		
Ch85 S– (M85 S–)		S–		
Ch99 S– (M99 S–)		S–		
Ladybirds				
M84d(F1-3) W– (Ch–)				W+
M70 W– (Ch–)				W–
M26(F1-4) W– (Ch–)				W+
M26(F1-6) W– (Ch–)			W–	
M84d(F1-8) W– (Ch–)			W+	
M84d(F1-9) W– (Ch–)			W–	
M19(F1-7) W– (Ch–)			W–	
M116(F1-4)♀ W– (Ch–)			W–	

Ch, *C. hippodamiae* mites; (Ch–) without mites; W, *Wolbachia*; S, *Spiroplasma*, "–" means the absence of infection, and "+" means the presence of infection.

contain double peaks. The mtDNA sequences of infected and uninfected with *Wolbachia* mites are identical. The results of comparing the DNA sequences of mites and ladybirds are presented in Fig. 3. Identical gene sequences of *Wolbachia* (alleles *cox*A-1 and *ftsZ*-3) were obtained for *A. bipunctata* M26(Ch90)W+, *A. decempunctata* M84d(F1-3)(Ch90)W+, and the donor Ch90W+. The same was obtained for *A. decempunctata* M84d(F1-8)(Ch109)W+ and the donor Ch109W+ (Table 3), in these cases, infection occurred with the strain *w*Abi-2 (Fig. 2).

A total of 58 beetles (without mites) were kept in Petri dishes with seven mite cultures (mite-infested ladybirds) (Appendix 1). In Table 3 only cases where ladybirds became parasitized with mites are shown. As a result, the mites became infected with *Wolbachia* and *Spiroplasma*; in three cases ladybirds not initially infected with *Wolbachia* acquired a symbiont after being bitten by mites.

DISCUSSION

C. hippodamiae mites were found on *A. bipunctata* imagoes in natural collections in Moscow, while *H. axyridis* imagoes were not infected. This indicates that it is *A. bipunctata* that continues to be the main host of *C. hippodamiae* in Moscow. It should be noted that there was a high level of infection, from 10.2 to 76.2% (Table 1), while in the years 1989–1997 in Moscow only 3.5–6.7% of *A. bipunctata* adults were infected (Zakharov and Eidelberg, 1997; Webberley et al., 2004). The geographic distribution of *C. hippodamiae* is increasing in Europe. Previously, it was found that *C. hippodamiae* was widely distributed in Central, Southern, and Eastern Europe, but absent from the northern and northwestern populations of *A. bipunctata* (Zakharov and Eidelberg, 1997; Webberley et al., 2006). Later, *C. hippodamiae* were found among *H. axyridis* in Poland (Rhule et al., 2010). In a population of the invasive ladybird species *H. axyridis* in the Netherlands, *C. hippodamiae* were not found in 2003–2007, but since 2008 *C. hippodamiae* has been found among wintering *H. axyridis* ladybirds (Raak-van den Berg et al., 2014). It is possible that for reproduction and distribution, *C. hippodamiae*, as well as many insects, is affected by an increase in the average annual temperatures.

Mite infestation of Coccinellidae by *C. hippodamiae* gradually leads to infertility of the female hosts. Eggs laid by infected females acquire a characteristic wrinkled appearance and dry out within a day after laying. It is assumed that infestation with mites prevents the formation of *A. bipunctata* chorion and this leads to shriveled eggs (Hurst et al., 1995). Experiments have shown that the viability of eggs laid by the hosts *A. bipunctata* (Hurst et al., 1995), *A. decempunctata* and *O. conglobata* (Webberley et al., 2004), and *H. axyridis* (Rhule et al., 2010) decreased markedly with the development of mite-borne infection. Our results also showed that immediately after mite infestation, most of the eggs laid by the mite-infested female remained fertilized. However, the percentage of hatched eggs began to decrease about ten days after infection. Similar results were obtained in other studies—a decrease in the proportion of hatched eggs at 10–15 days, which, as a rule, led to the sterility of females three weeks after infection (Hurst et al., 1995; Webberley et al., 2004). In experiments on *H. axyridis,* it was shown that in the first five days after infestation with mites, the frequency of hatching of larvae from eggs is more than 70%; around the 19th day there is a sharp decrease in hatching; and on the 30th day the hatching frequency is, on average, less than 20% (Rhule et al., 2010). These data suggest that mite infestation reduces ladybird fertility but does not always lead to absolute sterility.

According to our observations, mite infestation of *A. bipunctata* females leads to a decrease in fertility, but does not significantly affect the lifespan of ladybirds or their ability to mate. This has also been noted by other researchers (Webberley et al., 2004; Hurst et al., 1995). Thus, it can be assumed that, in the absence of stressful conditions, mites are able to live under the elytra of beetles for a long time, and this makes it possible for their bacterial symbionts to reach a high density.

Ladybirds are hosts to three hereditary symbiotic bacteria from the genera *Rickettsia, Wolbachia,* and *Spiroplasma*. We hypothesized that ectoparasites could be infected by these bacteria from ladybirds, which was confirmed by the results of experiments and the identity of the relevant sequences of the bacterial genes of *Wolbachia* and *Spiroplasma* from the ladybird hosts and their mites. We did not find *Rickettsia* in the studied insects. Mites become infected, inherit, and maintain the infection by *Wolbachia,* or by *Spiroplasma* over generations with the transition of mobile nymphs from the first ladybird host to other ladybirds. We observed the persistence of the infection status by *Wolbachia* and *Spiroplasma* over at least three life cycles (generations) of *C. hippodamiae.*

Can a mite infect a female ladybird with a microorganism? Our results showed that ladybirds from the *A. bipunctata* and *A. decempunctata* lines free from symbiotic bacteria become infected with *Wolbachia* after mite bites. We were unable to locate the transmission of *Wolbachia* to offspring from these beetles, since their eggs were not viable or the ladybirds themselves died without laying eggs. However, it should be noted that the fertility of ladybird eggs does not always drop to zero. In addition, since the frequency of hatching of eggs decreases gradually after infestation with mites, the female infested with mites has time to produce offspring, albeit not as numerous as compared to healthy females. In addition, female coccinellids can recover from the death of a mite colony (Hurst et al., 1995).

The efficiency of spread of bacterial symbionts among insects depends on the density and fitness of

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CLUSTAL format alignment by MAFFT (v7.490)

C.hippodamiae   ATAGTTGGAATATCCCTAAGAAATTTAATTCGAATAGAATTATCATCATCAAGACAAATT
A.bip_M26(Ch90) ATAGTAGGAACTTCTCTAAGAATTATTATTCGATTAGAATTAGGTACAACTAACAGACTA
                ***** ****. **.******* * * ****** ********    ** * *.  .* *

C.hippodamiae   ATCGGAGACCAACAAATTTACAACTCAATTGTAACATCACACGCATTCATTATAATCTTT
A.bip_M26(Ch90) ATTGGAAATGACCAAATTTATAATGTTATTGTAACAGCTCATGCTTTTATTATAATTTTT
                **.***.*. * ********.**. . ********* * **.** **.********.***

C.hippodamiae   TTCGTAATCATACCCATTATAATTGGAGGATTCGGTAACTGACTAATCCCATTAATATCA
A.bip_M26(Ch90) TTTATAGTTATACCCATTATAATTGGAGGTTTTGGAAACTGACTAGTACCTTTAATAATT
                **..**.*.******************** **.** *********.* ** ****** .

C.hippodamiae   ATAATACCTGATATAGCATTTCCACGTATAAACAATATAAGATTCTGAATACTATTAATA
A.bip_M26(Ch90) GGAGCGCCTGATATGGCATTCCCACGTCTTAATAATATAAGATTTTG---ATTATTACCT
                . *...********.*****.****** * **.***********.**   *.***** .

C.hippodamiae   TCAATATCAATACTATTAATATCTATAGTAACAGCAGAA---GGAACAGGAACTGGCTGA
A.bip_M26(Ch90) CCAGCTTTAACCCTTTTAATTTCTAGAAGAGTAATTGAAATGGGAGCAGGTACAGGATGA
                .**.. *.**. ** ***** **** *. *..*.. ***   ***.**** ** ** ***

C.hippodamiae   ACAATATACCCACCACTTTCAAGAAAT---CCCTTTCATGGACAATCCATAGATATAACT
A.bip_M26(Ch90) ACAGTATATCCACCTCTTTCTTCAAATATAGCACATAATGGGCCTTCTGTAGATTTAGTA
                ***.****.***** *****   ****    * . * ****.*  **..***** **..

C.hippodamiae   ATCCTTTCAATACACATAGCTGGAGTATCATCAATCATAAGCTCACTAAACTTTCTAGTA
A.bip_M26(Ch90) ATTTTTAGATTACACTTAGCTGGAATTTCATCAATTTTAGGAGCTGTAAATTTTATTTCT
                **..**  * ***** ********.* ********. **.*  *  ****.*** *  .

C.hippodamiae   TCTATTATCTCAATAACACCAAAAATAATAAAAGCTGAGCAGCTTCCTCTATTTAGATGA
A.bip_M26(Ch90) ACTATTATAAATATACGACCTAATGGGATAAATCTAGATAAAACACCTTTATTTGTTTGA
                 *******    ***  *** ** . .*****  . **  *. . ***.*****.  ***

C.hippodamiae   AGAATCATAATTACTACTCTACTACTAATTATAGCACTACCAGTTTTGGCAGGAGCAATT
A.bip_M26(Ch90) TCAGTTTTAATTACAGCTATTCTACTACTTTTATCATTACCAGTTCTTGCAGGAGCAATT
                  *.*. ******* .** * ****** ** ** **.********.* ************

C.hippodamiae   ACAATACTACTAACAGATCGAAACTGAAATACCTCATTCTTCGACCCAAGAGGAGGAGGA
A.bip_M26(Ch90) ACTATATTATTAACAGATCGTAATATTAATACATCATTTTTTGACCCTACTGGAGGAGGG
                ** ***.**.********** **.   ***** *****.**.***** *  ********.

C.hippodamiae   GACCCAATCCTATTCCAACATTTATTCTGATTTTTTGGTCACTGGAAAGGTTATAA
A.bip_M26(Ch90) GATCCAGTTTTATACCAACACTTATTTTGATTTTTTGGTCAC--------------
                **.***.*..*** ******.*****.***************
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Fig. 3. Comparison of *cox*1 gene sequences of the *Coccipolipus hippodamiae* mite and *Adalia bipunctata* ladybird.

the endosymbionts within the hosts. Thus, symbionts that cause cytoplasmic incompatibility reach, as a rule, a high density in the host population due to the obvious advantage of infected females. Male-killing bacteria benefit infected females by reducing inbreeding and allowing them to avoid starvation by feeding

on the nondeveloping eggs that males were supposed to hatch from. However, uninfected females and males are always present in the population. The prevalence of male-killing endosymbionts is much more sensitive to changes in the transmission fidelity and the relative fitness. In natural populations, symbionts that cause male-killing show much greater temporal and spatial variability in the infection prevalence than endosymbionts that cause cytoplasmic incompatibility. However, according to our data, infection by *Wolbachia* and *Spiroplasama* were stably preserved and inherited for at least three generations of both beetles and mites.

Could mites be vectors for the horizontal transfer of bacteria in nature? Strains of *Wolbachia* identical in the sequences of five genes were found in *A. bipunctata* *w*Abi-3 and *H. axyridis* kl-34 (Shaikevich et al., 2021). We watched how *H. axyridis* became infected with *C. hippodamiae* from *A. bipunctata* under experimental conditions. The vector of a symbiont between such phylogenetically distant species can be parasites, including *C. hippodamiae*. The ectoparasitic mites *C. hippodamiae* were previously studied only in connection with the possibility of their use for the control of the number of invasive predatory coccinellids *H. axyridis* (Rhule et al., 2010; Riddick, 2010). In this work, the infection of *C. hippodamiae* with bacterial symbionts from hosts was studied for the first time and their ability to spread bacteria among coccinellids has been proven.

CONCLUSIONS

Thus, as a result of this study, it was found that ectoparasitic *C. hippodamiae* mites become infected with the bacteria *Wolbachia* and *Spiroplasma* from the hosts and are capable of infecting ladybirds. The relationships between bacterial symbionts *Wolbachia* and *Spiroplasma* and the insect hosts vary from parasitism to mutualism. The long-term coexistence of symbiotic bacteria and their hosts presents great opportunities not only for sharing metabolic pathways, but also for the horizontal transfer of bacterial genes into insect genomes. In turn, horizontal gene influx through endosymbiosis is a source of new functions and may play a role in the evolution and symbiotic adaptation of hosts. In some cases, infection with new bacterial symbionts leads to the formation of reproductive barriers and, ultimately, to speciation. Thus, the study of the ways and means of horizontal transfers of inherited symbiotic bacteria among animals is of great importance both for the species studied and for other communities.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research, project no. 19-04-00739.

COMPLIANCE WITH ETHICAL STANDARDS

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

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at https://doi.org/10.1134/S1062359023700231.

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