SYMBIOGENETICS

Hereditary symbionts and mitochondria: distribution in insect populations and quasi-linkage of genetic markers

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Abstract

Maternal transmission ensures the joint transmission and simultaneous presence in populations of individuals with certain variants of the bacterial symbiont and host mitochondrial DNA. Such "quasi-linkage" of cytoplasmic genomes among insects and other arthropods is widespread. The symbiont acts as a "driver" of mitochondria and the obvious biological consequence is the spread of the "linked" mitochondrial haplotype in the population, which itself does not have increased selective value to the organism. Examples of such indirect selective mitochondrial sweep in insects are discussed, as well as biological consequences of this phenomenon and mechanisms of increasing the frequency of symbiont-infected individuals in the population.

Keywords: symbiogenome, cytoplasmic genomes, insects, hereditary symbiont, mitochondrial haplotype, co-transmission

Introduction

Hereditary symbionts (Lederberg, 1952; Preer, 1971) are symbiotic microorganisms, usually bacteria that live inside cells and persist during their divisions. In most cases, these microorganisms are not able to exist outside the host cells. They are classified into obligate microorganisms, necessary for maintaining the life of the macroorganism, and facultative ones, which infect only a part of individuals in the host population. Intracellular symbionts are especially common among insects, and they are also known in other arthropods.

Facultative hereditary symbionts in some cases have a well-marked effect on the host phenotype; in other cases, their effects are little or apparently absent. The presence of a symbiont in cells is usually detected by taking into account certain DNA markers characteristic of the genome of this symbiotic bacterium.

The interaction of the genomes of the symbiont and host at the genetic and functional levels allows them to be considered as a composite genome, as a single genetic system, aptly named "symbiogenome" (Tikhonovich and Provorov, 2010; Provorov and Tikhonovich, 2014).

Hereditary symbionts are not only preserved in daughter cells when an infected mother cell divides, they are also transmitted to offspring during reproduction. The study of how they are inherited in this case has shown that transmission almost always occurs in the female line, that is, from the mother to the offspring. It is also known that the mitochondrial genome is usually transmitted through the female line as well. Thus, there is a joint transfer of two parts of the symbiogenome — the symbiont genome and mitochondrial DNA (mtDNA), which can be traced by taking into account the corresponding markers of these two genomes. The joint transmission also ensures the joint presence in populations of individuals with certain variants of the symbiont and mtDNA.

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In the literature, for the phenomenon of joint transmission and simultaneous presence in populations of the corresponding variants of the genomes of the symbiont and mitochondria, a term borrowed from classical population genetics is used, "linkage disequilibrium". The authors suppose that the term "quasi-linkage", which will be used later in this article, will be more appropriate.

This paper presents the results of studies conducted on two subjects — mosquitoes (*Culex* and *Aedes*) and beetles of the *Adalia* genus. In conclusion, the data obtained by other researchers when studying various objects are briefly reviewed.

Mosquitoes

Coevolution of *Wolbachia* and host mtDNA in the *Culex pipiens* mosquito complex

The *Culex pipiens* complex currently includes five contemporarily recognized taxa: *Cx. quinquefasciatus, Cx. pipiens, Cx. pipiens pallens, Cx. australicus* and *Cx. globocoxitus*. *Cx. australicus* and *Cx. globocoxitus* are Australian endemic taxa. *Cx. p. pallens* is endemic in Japan. *Cx. quinguefasciatus* is widely distributed in tropical and subtropical regions, and *Cx. pipiens* is ubiquitous in temperate regions. The latter species includes mosquitoes of the *pipiens* and *molestus* forms with pronounced behavioral and physiological characteristics (Fedorova and Shaikevich, 2007). Mosquitoes of the form *molestus* are autogenous (first oviposition without a prior blood meal), stenogamous (able to mate in a narrow space and do not form a swarm), and anthropophilous (prefer biting mammals, including humans), and they do not have winter diapause. On the contrary, mosquitoes of the form *pipiens* are anautogenous (require a blood meal for egg development), eurygamous (need a lot of space for mating, swarming), diapause-forming and ornithophilic (they prefer biting birds). In temperate regions, *Cx. pipiens* f. *molestus* mostly reproduce in the basements of urban apartment buildings (Vinogradova et al., 2007), but they breed aboveground in the Mediterranean region (Gomes et al., 2009). Mosquitoes of the tropical subspecies *Cx. quinguefasciatus* are anautogenous, stenogamous, anthropophilous, and have no diapause.

Cx. torrentium is a cryptic species, closely related to *Cx. pipiens* (Fedorova and Shaikevich, 2007). Vinogradova (see Shaikevich et al., 2006) has successfully crossed mosquitoes in the laboratory; hybrids had nuclear DNA from both *Cx. pipiens* and *Cx. torrentium*. In nature, hybrids between two species were not found (Fedorova and Shaikevich, 2007). *Cx. torrentium* and *Cx. pipiens* f. *pipiens* have similar biological features: both are non-autogenous, diapausing, and eurygamous. *Cx. torrentium* is widely distributed in the Palearctic, and both species often inhabit the same water bodies. Only minor morphological differences exist in their male genitalia. *Cx. torrentium* and *Cx. pipiens* originated from a common ancestor based on the phylogenetic analyses (Shaikevich and Zakharov, 2010; Dumas et al., 2016).

Mosquitoes of the *Culex pipiens* complex were characterized by the coinheritance of mtDNA and symbiotic bacteria *Wolbachia* (Rasgon et al., 2006; Dumas et al., 2013; Shaikevich and Zakharov, 2014; Shaikevich et al., 2016).

MITOCHONDRIAL DNA DIVERSITY IN *WOLBACHIA*-INFECTED AND UNINFECTED *CULEX* MOSQUITOES

The sequence diversity of the mitochondrial nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit 4 (ND4) gene was investigated in *Wolbachia*-infected (South Africa (SA), California and Thailand) and uninfected (SA) *Culex pipiens* s.l. populations. In total, 12 mtDNA haplotypes (A–L) were identified. In infected populations, 99 % of individuals had a single haplotype K. In the uninfected SA population, 11 haplotypes were present, including K (Rasgon et al., 2006). Recently, three new mtDNA haplogroups were found in *Wolbachia*-uninfected specimens, and a different one in *Wolbachia*-infected mosquitoes within the *Culex pipiens* mosquito complex (Dumas et al., 2016). Concatenated sequences of the NADH dehydrogenase subunit 2 (*ND*2), subunit 4 (*ND*4) and cytochrome oxidase I (*COI*) genes of mtDNA were analyzed (Dumas et al., 2016).

The polymorphism of the full-size DNA sequence of the *COI* gene (1548 bp) was studied in *Wolbachia*-infected *Culex pipiens* f. *pipiens* and f. *molestus* and *Wolbachia*-uninfected *Cx. torrentium* from 16 locations in Russia and in 3 laboratory strains of subtropical *Wolbachia*-infected *Cx. quinguefasciatus* and *Cx. pallens* (Shaikevich and Zakharov, 2010). In 16 populations of the studied mosquito species, 15 haplotypes were found (A–O); 10 of these were detected in the *Wolbachia*-uninfected population of *Cx. torrentium*. Three haplotypes were detected for *Cx. pipiens* f. *pipiens* (A, B, C); these haplotypes were not population-specific (Fig. 1). *Cx. p. pallens,* which is considered a variety of *Cx. pipiens* (Harbach, 2012), have a *COI* sequence identical with the main haplotype of *Cx.* f. *pipiens* (A). *Cx. quinquefasciatus* have haplotypes E and E1. Monomorphic haplotypes were found in *Cx. pipiens* f. *molestus* (D) (Fig. 1). The number of haplotypes in *Cx. pipiens* was low, and the same haplotypes were found in distant populations.

Based on the DNA variability of the *COI* gene of both forms of *Cx. pipiens* and *Cx. Quinquefasciatus,* we developed restriction fragment length polymorphism (PCR-RFLP) assays (Shaikevich, 2007; Shaikevich, 2009). *Hae*III digestion of the *COI* PCR products allowed the discrimination of the D haplotype from A, B,

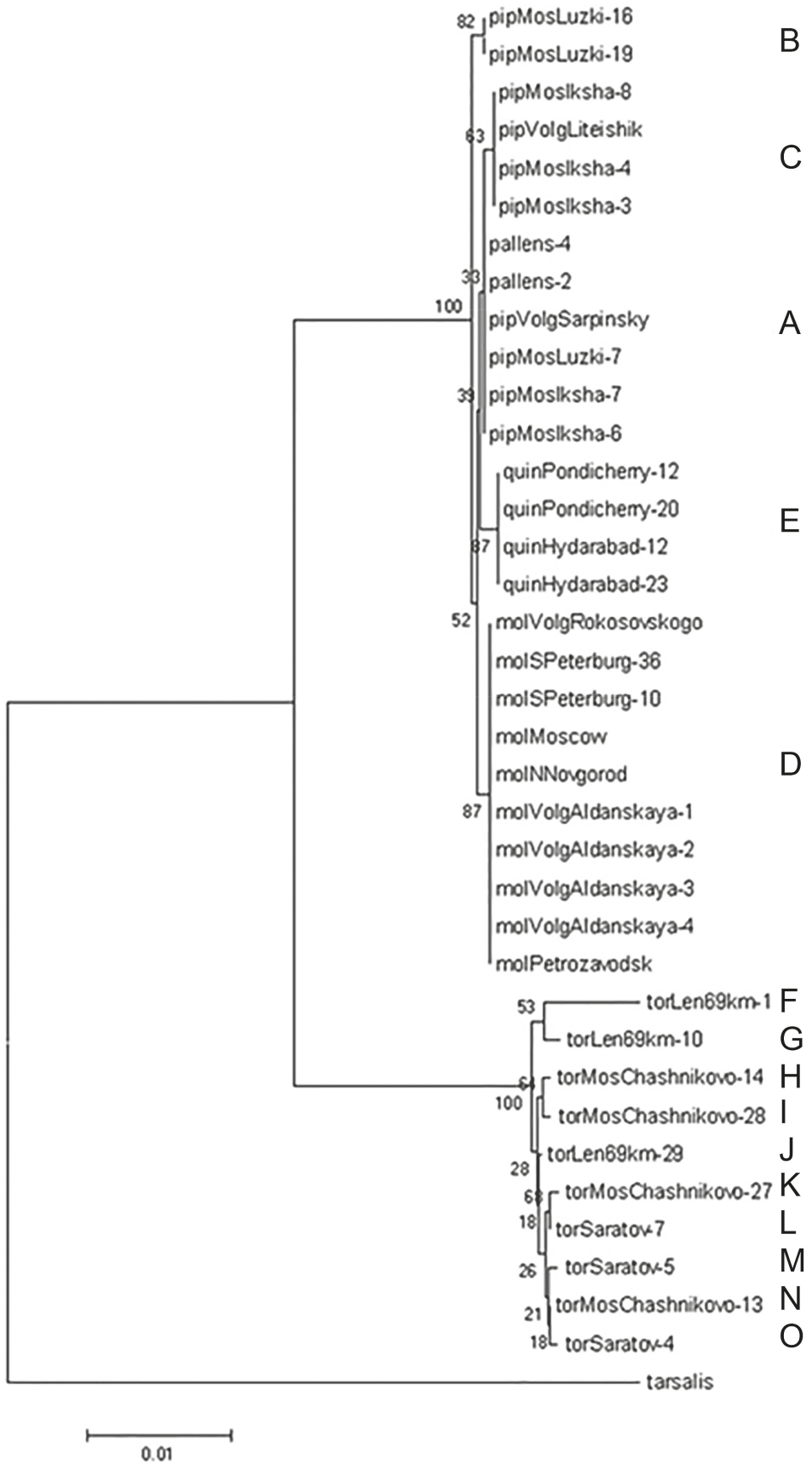

Fig. 1. The similarity dendrogram obtained for nucleotide sequences of 1548 bp of the gene *COI* from mosquitoes of the *Cx. pipiens* complex (Shaikevich and Zakharov, 2010): quin — *Cx. quinquefasciatus*, pallens — *Cx. pallens*, pip — *Cx. pipiens* f. *pipiens*, mol — *Cx. pipiens* f. *molestus*) and *Cx. torrentium* (tor). The haplotypes are marked by letters on the right. Mos — Moscow, Volg — Volgograd, Len69 — Leningrad obl., Pondichery and Hydarabad — origin of *Cx. quinquefasciatus* (India). *C. tarsalis* (AF425847) was used as an external reference.

C and E haplotypes. *Alu*I allows the discrimination of E and E1 haplotypes from others. Haplotypes A, B and C cannot be differentiated using the PCR-RFLP approach, but A, B and C haplotypes differ from each other by one SNP and were found only in *Cx. pipiens* f. *pipiens*. This made it possible to combine them into haplogroup A (Shaikevich and Zakharov, 2014). Similarly, haplotypes E and E1 are close relatives and were found predominantly in *Cx. quinquefasciatus*, and we put them together into haplogroup E (Shaikevich et al., 2016). Monomorphic haplotype D was found in *Cx. pipiens* f. *molestus* (Shaikevich and Zakharov, 2010; Shaikevich and Zakharov, 2014; Shaikevich et al., 2016).

At the same time, *Cx. torrentium* have numerous haplotypes, and each studied individual has a unique haplotype (Shaikevich and Zakharov, 2010). The data show a considerable decrease in mtDNA polymorphism in the *Wolbachia*–infected population of *Cx. pipiens* compared with uninfected *Cx. torrentium* (Table 1). These data correspond to the observations of Rasgon et al. (2006) that *Wolbachia*-uninfected *Culex* mosquitoes have high mtDNA diversity. Polymorphism in the nuclear sequence (ITS2) was similar in infected and uninfected *Cx. pipiens* (Rasgon et al., 2006; Dumas et al., 2016), as well as in infected *Cx. pipiens* and uninfected *Cx. torrentium* (Shaikevich and Zakharov, 2010). This suggests that the reduction of mitochondrial polymorphism is more likely to be due to the selective sweep of the *Wolbachia*-infected haplotype than to a passage of *Cx. pipiens* through a bottleneck before the spread around the world.

Table 1. Diversity in *Wolbachia*-infected and uninfected *Culex* based on *COI* sequence polymorphism

Species	*Wolbachia*	N	h	S	Pi	K
Cx.pipiens f.*pipiens*	98 %	10	3	3	0.0008	1.24
Cx.pipiens f.*molestus*	100 %	10	0	0	0	0
Cx.torrentium	0 %	10	10	21	0.00346	5.4

N. — studied specimens, h — number of haplotypes, S — number of polymorphic sites, Pi — nucleotide diversity, K — average number of nucleotide differences

The influence of infection on the fitness of *Cx. pipiens* is difficult to assess, since the infection rate in natural populations of this species is almost 100 % (Shaikevich and Zakharov, 2010; Atyame et al., 2011; Dumas et al., 2016; Shaikevich et al., 2016). However, in laboratory experiments it was demonstrated that *Wolbachia*-infected *Cx. quinquefasciatus* females develop and lay their eggs earlier compared to the cured mosquitoes. This effect and induction of CI were found to be reproductively advantageous to infected females, compared to aposymbiotic ones (Almeida et al., 2011).

WOLBACHIA VARIABILITY AND ASSOCIATION WITH MTDNA IN *CULEX PIPIENS*

Wolbachia from *Culex pipiens* s.l. isolates collected in different geographical regions have identical MLST profiles and *wsp* sequences (Shaikevich et al., 2019). However, detailed analysis that included some other genes, coding proteins with ankyrin (ANK) domains (Atyame et al., 2011), revealed the genogeographic specificity of bacterial strains (Dumas et al., 2013; Shaikevich et al., 2016). *Wolbachia* induces cytoplasmic incompatibility (CI) in *Cx. pipiens* (Ghelelovitch, 1952; Laven, 1967; Atyame et al., 2014). The unidirectional CI phenomenon is embryonic mortality of offspring when an infected male mates with an uninfected female; the bidirectional CI is when the male and female are infected with different strains (Yen and Barr, 1971; Werren et al., 2008; Atyame et al., 2014). Five genetically different groups of *Wolbachia* (*w*Pip) show different incompatibility status (Atyame et al., 2014).

In Russia, three different *w*Pip groups were identified in *Culex pipiens* s.l. (Shaikevich and Zakharov, 2014). More haplotypes and *w*Pip groups were detected in *Cx. pipiens* s.l. populations in the Mediterranean region (Shaikevich et al., 2016). Culex mtDNA of 580 specimens from 35 geographical populations was genotyped using a series of specific PCR-RFLP assays (Shaikevich et al., 2016). The association between the *COI* haplogroup and the *w*Pip group shows the co-transmission of cytoplasmic components (Table 2).

A strong association was observed between the *COI* D haplotype, as well as the A and E haplogroups and *w*Pip groups and taxa (Chi-square = 732.71, d.f. = 8, $P < 0.0001$) in 35 distinct geographical populations of the *Culex pipiens* complex. Haplogroup A and infection with *w*PipII and *w*PipIII appear to be typical for *Cx. pipiens* f. *pipiens* (Table 3). Haplotype D and infection with *w*PipIV are characteristic of the f. *molestus*, while haplogroup E, characteristic of *Cx. quinquefasciatus*, was correlated with *w*PipI (Shaikevich et al., 2016). Infection of *Cx. quinquefasciatus* with bacteria of the *w*Pip-I group and *Cx. pipiens* (f. pipiens) with bacteria of the *w*Pip-II and *w*Pip-III groups was found in a study of 823 mosquitoes from around the world with high statistical reliability (Fisher exact test, $P < 10^{-4}$) (Dumas et al., 2013).

Hybridization events in *Cx. pipiens* populations in coastal regions of Southern Europe and North Africa lead to the mixing of cytoplasmic components among taxa (Shaikevich and Vinogradova, 2014; Shaikevich et al., 2016). Therefore, the pattern of mtDNA variation reflects the evolutionary history of *w*Pip infection rather than of the mosquito taxa in the Mediterranean region (Dumas et al., 2013). Nevertheless, a statistically significant correlation between the *COI* haplogroup and taxa has been observed also in sympatric populations after

Table 2. The association between mtDNA of *Culex pipiens* s.l. and type of bacteria *Wolbachia*

Sampling place	N	*COI* type**	wPip*** group	References
Russia, Krasnodar	6	A	II	Shaikevich et al., 2016
Russia, Mos. obl	16	A	II	Shaikevich and Zakharov, 2014
Russia, Moscow*	20	D	IV	Shaikevich et al., 2016
Russia, S-Peterburg1*	8	D	IV	Shaikevich et al., 2016
Russia, S-Peterburg2*	7	D	IV	Shaikevich et al., 2016
Russia, Ekaterinburg*	6	D	IV	Duma et al., 2013
Russia, Tomsk*	9	D	IV	Duma et al., 2013
Russia, Volgograd	12	A	II	Shaikevich et al., 2016
Russia, Volgograd	7	A	III	Shaikevich and Zakharov, 2014
Russia, Volgograd*	8	D	IV	Shaikevich et al., 2016
Kazakhstan, Almaty	21	D	IV	Shaikevich and Zakharov, 2014
Tunis, Nefza	16	D	IV	Shaikevich et al., 2016
Tunis, Tabarka	12	D	IV	Shaikevich et al., 2016
Morocco, Tanger	1	E	I	Shaikevich et al., 2016
Morocco, Casablanca	2	A	II	Shaikevich et al., 2016
Greece, Kos	24	E	I	Shaikevich & Vinogradova, 2013
Italy, Piedmont	18	D	IV	Shaikevich et al., 2016
Portugal, Comporta	4	E	I	Shaikevich et al., 2016
Portugal, Comporta	6	E	I	Shaikevich et al., 2016
Germany, Berlin*	4	D	IV	Shaikevich et al., 2016
Germany, Berlin	9	A	II	Shaikevich et al., 2016
Germany, Hannover*	1	D	IV	Shaikevich et al., 2016
Germany, Hannover	17	A	II	Shaikevich et al., 2016
France, Prades-le-Lez 1	16	A	II	Shaikevich et al., 2016
France, Prades-le-Lez 2	22	A	II	Shaikevich et al., 2016
France, Saint-Nazaire de Pezan	12	A	II	Shaikevich et al., 2016
T7 strain, France,Montpellier	11	A	II	Shaikevich et al., 2016
Pondicherry, India	23	E	I	Shaikevich and Zakharov, 2014
Hyderabad, India	20	E	I	Shaikevich and Zakharov, 2014
Total	338			

* undergound (or indoor) sampling sites; **based on PCR-RFLP analysis group A includes A, B and C haplotypes, group E includes E and E1 haplotypes (Shaikevich et al., 2016); ***based on analysis of ank2 and pka1 *Wolbachia* genes

the confirmation of *Cx. pipiens* taxonomy status by microsatellite (CQ11) assay (Shaikevich et al., 2016). Fixed nucleotide substitutions in the *COI* gene that can distinguish the complex members were found in allopatric *Cx. pipiens* f. *pipiens* and f. *molestus* populations in a temperate climate, as well as between temperate *Cx. pipiens* and tropical *Cx. quinquefasciatus* (Shaikevich and Zakharov, 2010; Danabalan et al., 2012; Gunay et al., 2015; Shaikevich et al., 2016). The existing subspecies-specific pattern of mtDNA haplotypes and its association with the *w*Pip group among *Wolbachia*-infected populations of mosquitoes *Cx. pipiens* s.l. suggest that the infection of mosquitoes with the endosymbiotic bacteria occurred earlier than the divergence of mitochondrial DNA and taxa. Probably, bacterial infection played a key role in subspecies formation, since it is known that *w*Pip induc-

Table 3. Partitioning of *COI* haplotypes between *Cx. pipiens* taxa

COI type	N	Cx. pipiens taxa			
		Cx. pipiens f. *pipiens*	*Cx. pipiens* f. *molestus*	*Cx. quinquefasciatus*	*Cx. pipiens*/*Cx. quinquefasciatus* hybrid
Group A	222	201 (91 %)	11 (5 %)	0	10* (4 %)
D	220	20 (9 %)	179 (81 %)	0	21* (10 %)
Group E	125	10*	10*	43	0

* only in Mediterranean population

es different cytoplasmic incompatibility patterns in *Cx. pipiens* (Atyame et al., 2014; Altinli et al., 2018).

Wolbachia variability and association with mtDNA in *Aedes albopictus*

Two *Wolbachia* strains, *w*AlbA and *w*AlbB, coinfect nearly all studied *Ae. albopictus*. *Wolbachia*-infected *Ae. albopictus* females were observed to have increased longevity, greater oviposition rates, and higher egg hatch rates and an increased fecundity (Dobson et al., 2002). *Wolbachia* induces cytoplasmic incompatibility in *Ae. albopictus* (Sinkins et al., 1995; Dobson et al., 2004). Most populations are naturally infected with both strains, suggesting that superinfection is common in the field-collected *Ae. albopictus* worldwide (Armbruster et al., 2003; Ahmad et al., 2017). The frequency of *Wolbachia* infection in most *Ae. albopictus* populations tends to 100 %, but it varies in certain populations. On the Russian Caucasus coast, both *w*AlbA and *w*AlbB strains were found in *Ae. albopictus*, collected in 2011, 2012, 2013 and 2016 (Shaikevich et al., 2018). Of the 411 studied individuals, 56 % were *Wolbachia*-positive. Among 234 infected specimens, 3 variants of infection were found: rare strain *w*AlbA (1.7 %), common strain *w*AlbB (78.6 %), and superinfection with both strains *w*AlbA and *w*AlbB (19.7 %); 177 specimens were not infected. Among them, 159 mosquitoes were *Wolbachia*-free in one collection in 2016 from Dagomys. Good PCR results for the other mosquito genes ruled out the poor quality of mosquito DNA in this collection (Shaikevich et al., 2018). The frequency of *Wolbachia* infections was recently investigated in 14 *Ae. albopictus* populations in China (Guo et al., 2018). Most positive individuals were infected with both the *w*AlbA and *w*AlbB strains of *Wolbachia*. The infection rate in eight populations was 100 %. But, in two of the *Ae. albopictus* populations, only 50 % and 60 % of individuals were infected with *Wolbahia* (Guo et al., 2018).

In contrast to *Culex pipiens*, high mtDNA polymorphism is observed in *Ae. albopictus* populations, especially in the areas of the native range of the species in Southeast Asia (Poretta et al., 2012; Fang et al., 2018; Ruling et al., 2018). Some previous studies of *Ae. albopictus* populations supposed a low genetic diversity in mitochondrial markers (Ambrurster et al., 2003 and reference therein). This was later refuted (Poretta et al., 2012; Battaglia et al., 2016): the reasons for the low genetic diversity were that 1) in the first studies, most *Ae. albopictus* mitochondrial DNA surveys were restricted to short segments of the *COI* and/or NADH dehydrogenase subunit 5 (ND5) genes; 2) laboratory stocks or sibling eggs were included in these studies. Hundreds of haplotypes were identified after the analyses of almost the entire *COI* gene (90 % of the entire *COI* gene in length) both in native and colonized areas (Porreta et al., 2012; Zhong et al., 2013; Giordano et al., 2019; Hu et al., 2020; Zé-Zé et al., 2020). No co-evolution of mtDNA haplotypes and *w*Alb strains or any loss of mtDNA diversity in infected *Ae. albopictus* individuals was revealed (Minard et al., 2017; Guo et al., 2018).

If one compares *Wolbachia*-infected *Ae. albopictus* and uninfected representatives of cryptic species, the latter demonstrate greater variability of mtDNA in China (Guo et al., 2018), but not in Vietnam (Minard et al., 2017). In Southeast Asia, sympatric cryptic species have been identified in the *Ae. albopictus* subgroup (Minard et al., 2017; Guo et al., 2018). *Ae. albopictus* sibling species are combined in the literature as members of the *Scutellaris* group and *Ae. albopictus* subgroup. These species have very similar morphological characteristics, especially at the larval and adult (females) stages. Furthermore, although some of the species of this subgroup have different ecological niches, some of them are found in sympatry (Minard et al., 2017 and the reference therein). Contrary to *Ae. albopictus*, the cryptic species did not harbor any *Wolbachia* infection in Vietnam. However, individuals of cryptic species presented similar haplotype diversity relative to *Ae. albopictus* in Vietnam (Minard et al., 2017). Another pattern was observed in the cryptic species in China (Guo et al., 2018). *Wolbachia* was rare but was present in populations of cryptic species in China. A total of 47 *COI* haplotypes were detected in 129 *Ae. albopictus* mosquitoes and 10 haplotypes derived from 11 specimens of cryptic species, indicating significantly higher genetic diversity found in cryptic species populations than in *Ae. albopictus* populations in China (Guo et al., 2018).

Conclusion

In the *Culex pipiens* complex and in the cryptic species *Cx. torrentium*, uninfected individuals show higher mitochondrial diversity than infected ones (Rasgon et al., 2006; Shaikevich and Zakharov, 2010; Atyame et al., 2011; Dumas et al., 2016). Indeed, non-infected populations of *Cx. pipiens* harbored a higher ancestral mitochondrial diversity (Rasgon et al., 2006). The initial *w*Pip sweep through *Cx. pipiens* s.l. is estimated to have occured 20,000 years ago (Dumas et al., 2016) or up to 47,000 years ago (Rasgon et al., 2006). This indicates that *w*Pip invasion in the *Cx. pipiens* complex was recent and rapid. The invasion of the *w*Pip ancestor resulted in an indirect selective mitochondrial sweep due to CI, which led to the loss of mtDNA variation within *Cx. pipiens* populations (Rasgon et al., 2006; Dumas et al., 2016).

Such mitochondrial sweep was not supported in *Ae. albopictus*, as no significant differences in the haplotype diversities were observed between *Ae. albopictus* and the cryptic species (Minard et al., 2017). High genetic diversity is typical for individuals of *Ae. albopictus* infected with *w*Alb in the native range (Ruling et al., 2018). The diversity of mtDNA in *Ae. albopictus* is reduced in some invasive populations due to the founder effect, unrelated to *Wolbachia* infection. The fact that individuals with different mitochondrial haplotypes are infected with the same *w*AlbA and *w*AlbB strains indicates that mtDNA diversity arose after the initial infection. Moreover, recombination between primarily monophyletic *w*AlbA and *w*AlbB strains was observed in *Ae. albopictus* (Shaikevich et al., 2019). These facts, together with the wide diversity of mtDNA haplotypes, indicate an ancient infection in *Ae. albopictus*.

Ladybirds

Beetles of the *Adalia* genus (Coleoptera: Coccinellidae) have been the subject of research in the field of ecological genetics for a long time due to their wide distribution and easily registered morphological polymorphism. In the 2000s, the study of their molecular polymorphism began. When analyzing the nucleotide diversity of the *COI* mitochondrial gene in *Adalia bipunctata*, 17 mitotypes were described (Shaikevich and Zakharov, 2015). Most of them differ by 1–4 nucleotides; however, two mitotypes, H9 and H10, differ from the others at the interspecies level (Schulenburg et al., 2002; Shaikevich and Zakharov, 2015); they may have been included in the gene pool of *A. bipunctata* as a result of ancient hybridization with an unknown (extinct?) species.

Studying the hybridization of beetles of different populations, Lus (1947) discovered the existence of females giving only female offspring. This feature turned out to be an inherited trait passed strictly through the female line. Forty-five years after the discovery of this phenomenon, its nature was deciphered — it turned out that the one-sex female offspring was the result of the death of male embryos, which is caused by symbiotic bacteria living in the cytoplasm (Werren et al., 1994). DNA analysis allowed identification of these bacteria. The same effect, the death of male offspring, is caused by three different bacteria: *Rickettsia*, *Wolbachia* and *Spiroplasma*. Beetles infected with different bacteria may be present in the same population (Majerus et al., 2000).

Since the same population of ladybirds may contain individuals infected with different female-transmitted bacteria, the relationship between the presence of a particular bacterium and the host mitotype has been studied. In the work by Schulenburg et al. (2002) (with the participation of one of the authors of this article), a correlation between bacterial infection and the mtDNA type was shown.

Rickettsia was found in individuals of mitotypes H9 and H10 (one individual had mitotype H7); *Wolbachia* — in individuals of mitotypes H1(one strain of this bacterium), H3, H6, H8 (another strain); *Spiroplasma* — in individuals of mitotypes H1, H2. In this study, beetles from populations of Great Britain, Germany, Denmark and Russia (the majority of individuals from St. Petersburg and Moscow) were studied. The authors continued this research by using material from a broader geographical origin: from Moscow, St. Petersburg, Arkhangelsk, Karelia, Buryatia, and other places (Zakharov and Shaikevich, 2011; Shaikevich and Zakharov, 2015).

Rickettsia was found in individuals of the H10 mitotype (a total of 19 individuals from 3 populations); *Spiroplasma* — in 19 individuals from 2 populations with the H1, H2, H3, H11 and H17 mitotypes. It should be noted that the H2, H3, H11 and H17 mitotypes are related to the H1 mitotype and differ from it by one nucleotide each.

What can be concluded from the above data?

The wide geographical distribution of two symbiotic bacteria (*Rickettsia* and *Spiroplasma*), with a clear "coupling" of infection with certain mitotypes, indicates that infection is a rare event and it occurred long ago, at least before the end of the last ice age. The prescription of the act of infection of ladybirds with *Spiroplasma* bacteria is also indicated by the fact that the bacterium occurs in individuals with several, but related mitotypes — that is, mutations-replacements of single nucleotides that led to the appearance of the 2, 3, 11 and 17 mitotypes occurred after the act of infection. This is also evidenced by the presence of *Rickettsia* in a ladybird (mitotype H10) from Buryatia, where, as is known, there is a special subspecies, *A. bipunctata fasciatopunctata*; that is, differentiation into subspecies occurred after infection with *Rickettsia* of the ancestral population of beetles.

The horizontal transfer of bacteria, if it occurs, is rare. It can only be described by a single case of finding

Rickettsia in a beetle not of the H9 or H10 mitotypes, but of the H7 mitotype, which is not related to the first two (Schulenburg et al., 2002).

In individuals of another species, *A. decempunctata*, only *Rickettsia* (Schulenburg et al., 2001; Zakharov and Shaikevich, 2001) was found from symbiotic bacteria. Eight mitotypes were identified in this species (Shaikevich et al., 2019), but no association of mitotypes and *Rickettsia* infection was found.

Symbionts of different arthropods

The first or one of the first studies to demonstrate the relationship of a symbiont with a specific variant of mtDNA was a study that examined the diversity of mtDNA in individuals of *D. simulans* infected and uninfected with *Wolbachia*. Individuals infected with *Wolbachia* from populations from California (USA), Canada, Italy and South Africa all had the same mitotype (B), while among the uninfected individuals, two other mitotypes were identified along with mitotype B (Hale and Hoffmann, 1990).

The fast (within a few years) spread of *Wolbachia*, which causes the phenomenon of cytoplasmic incompatibility, was shown in the study of populations of this species of *Drosophila* from California. Infected flies had a single mitotype. Uninfected individuals from the same populations showed high mtDNA polymorphism (Turelli et al., 1992).

Ilinsky (2013) studied a large number of *Drosophila melanogaster* lines of different geographical origin and revealed two main mitotype clades: *M* and *S,* each being strictly associated with one of the two major *Wolbachia* groups, MEL and CS, respectively (see also Bykov et al., 2019). No evidence of horizontal transmission of *Wolbachia* between maternal lineages has been found.

In the terrestrial crustacean *Armadillidium vulgare*, 11 mitotypes were detected; in individuals from different populations infected with *Wolbachia*, which causes the feminization of genetically male individuals, only 2 of the 11 were found. These two mitotypes, Av1 and Av2, were closely related (Rigaud et al., 1999).

Populations of *Acraea encedon* and *A. encedana* butterflies consist of individuals infected with *Wolbachia*, which in these species causes the death of male offspring and uninfected individuals. The first species is infected with two strains of *Wolbachia*; the second, with one species. Nine mitotypes are variously represented among infected and uninfected individuals. The UG mitotype is found among infected individuals of both species in populations of Ghana, Tanzania, Uganda and Zimbabwe, as well as among uninfected individuals of both species from Uganda. The TZ mitotype is found only in infected *A. encedon* from Tanzania; the remaining seven mitotypes were found only among uninfected individuals of *A. encedon*. It can be concluded that the symbiont is maintained in populations of *A. encedon* linked to one mitotype and the same pair of genomes as a result of introgression during interspecific hybridization was transferred to the gene pool of the second species (Jiggins, 2003).

General conclusion

The materials presented in this article show a wide spread of quasi-linkage of cytoplasmic genomes among insects and other arthropods. Since the transfer of intracellular symbionts usually occurs through female gametes, the phenomenon discussed should occur in all cases where there is no or very rare horizontal transmission of the symbiont. A discussion of the consequences of quasi-linkage of mitochondrial genomes and cytoplasmic symbionts is given in the article by Johnstone and Hurst (1996), see also Perlman et al. (2015).

What are the biological consequences of this phenomenon?

The first obvious consequence is the spread of a special mitotype in the population, which itself does not have increased selective value. The symbiont acts as a "driver" and in cases when the symbiont spreads in the population, the "linked" mitotype also spreads. There are two mechanisms of increasing the frequency of symbiont-infected individuals in the population: the symbiont manipulates host reproduction, causing the domination of infected individuals in the offspring, and the symbiont increases the fitness of infected individuals in comparison with uninfected ones (as a result of increased resistance to parasites, increased lifespan, etc.). The distribution of *Wolbachia* in California populations of *Drosophila simulans* and the simultaneous distribution of a special mitotype, not previously encountered in these populations, illustrates the phenomenon (Turelli et al., 1992).

It is possible to assume the coadaptation of the "linked" cytoplasmic genomes — both the genome of a symbiont that found itself in a new cell environment and the mitochondrial genome, since the presence of a large number of bacteria in the cytoplasm must affect the energy balance of the cell. It is difficult to detect such coadaptation. Possibly an increase in the frequency of mutations in mtDNA in *Wolbachia*-infected lines may reflect the process of coadaptation of cytoplasmic genomes (Shoemaker et al., 2004).

Finally, species of the Lepidoptera order have heterogametic female sex. As a result, three genomes are linked — the W chromosome, mtDNA, and the symbiont genome. The W chromosome must also be involved in the coadaptation process along with the other two genomes.

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