Culex quinquefasciatus mosquitoes do not support replication of Zika virus
Ricardo Lourenço-De-Oliveira, João T. Marques, Vattipally Sreenu, Célestine Atyame Nten, Eric Roberto Guimarães Rocha Aguiar, Margus Varjak, Alain Kohl, Anna-Bella Failloux

To cite this version:
Ricardo Lourenço-De-Oliveira, João T. Marques, Vattipally Sreenu, Célestine Atyame Nten, Eric Roberto Guimarães Rocha Aguiar, et al.. Culex quinquefasciatus mosquitoes do not support replication of Zika virus. Journal of General Virology, Microbiology Society, 2017, <10.1099/jgv.0.000949>. <pasteur-01677759>

HAL Id: pasteur-01677759
https://hal-pasteur.archives-ouvertes.fr/pasteur-01677759
Submitted on 8 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution 4.0 International License
Culex quinquefasciatus mosquitoes do not support replication of Zika virus

Ricardo Lourenço-De-Oliveira, Jo Ao, T Marques, Vattipally Sreenu, C Elestine, Atyame Nten, † Eric, Roberto Guimar, Rocha Aguiar, Margus Varjak, et al.

To cite this version:

Ricardo Lourenço-De-Oliveira, Jo Ao, T Marques, Vattipally Sreenu, C Elestine, et al.. Culex quinquefasciatus mosquitoes do not support replication of Zika virus. Journal of General Virology, Microbiology Society, 2017, <10.1099/jgv.0.000949>. <pasteur-01677759>
Culex quinquefasciatus mosquitoes do not support replication of Zika virus

Ricardo Lourenço-de-Oliveira,1,2 João T. Marques,3 Vattipally B. Sreenu,4 Célestine Atyame Nten,1† Eric Roberto Guimarães Rocha Aguiar,3 Margus Varjak,4 Alain Kohl* and Anna-Bella Failloux1*  

Abstract

The rapid spread of Zika virus (ZIKV) in the Americas raised many questions about the role of Culex quinquefasciatus mosquitoes in transmission, in addition to the key role played by the vector Aedes aegypti. Here we analysed the competence of Cx. quinquefasciatus (with or without Wolbachia endosymbionts) for a ZIKV isolate. We also examined the induction of RNA interference pathways after viral challenge and the production of small virus-derived RNAs. We did not observe any infection nor such small virus-derived RNAs, regardless of the presence or absence of Wolbachia. Thus, Cx. quinquefasciatus does not support ZIKV replication and Wolbachia is not involved in producing this phenotype. In short, these mosquitoes are very unlikely to play a role in transmission of ZIKV.

Zika virus (ZIKV) emerged in Yap Island in Micronesia and then in French Polynesia in 2013–2014, and after affecting most of the South Pacific islands, the virus was eventually detected in the Americas in 2015 [1–4]. The increased number of infections in humans included cases with unusually severe symptoms such as Guillain–Barré syndrome and developmental abnormalities in newborns that are now described as congenital Zika syndrome [5–8]. As there are currently no licensed vaccines or specific therapies, the only way to interrupt ZIKV transmission is by controlling mosquito populations [9]. An enzootic cycle limited to Africa and Asia with occasional spillover events has been described [10, 11]. In the current outbreak in the Americas, ZIKV is believed to be mainly transmitted by the human-biting mosquito Aedes aegypti [12, 13]. Whether a similar enzootic cycle in the Americas can be established is not known and it has been suggested that such a process would make eradication efforts “practically impossible” [14]. Experimental infections with the epidemic ZIKV genotypes demonstrated that populations of Ae. aegypti and Ae. albopictus mosquitoes, which are associated with a risk of local transmission [15] as well other aedine species were heterogeneously and weakly competent for transmission [16–25]. Therefore, the potential role of other anthropophilic mosquitoes in the ZIKV outbreak raised a question which took time to be addressed [26]. Culex quinquefasciatus Say is an opportunistic blood feeder predominant in urban settings throughout the tropics where it is frequently the most annoying biting pest to humans [27]. This night-active mosquito is also the vector of many pathogens including arboviruses such as West Nile and St. Louis encephalitis viruses [28], which belong to the genus Flavivirus of the family Flaviviridae and are related to ZIKV. Both Cx. quinquefasciatus and Culex pipiens (from temperate regions) mosquitoes of the Cx. pipiens complex were not able to experimentally transmit ZIKV and no viral particles were detected in mosquito saliva up to 21 days after exposure to an infectious blood meal [17–20, 25, 29–36], though different results were recorded with other Cx. quinquefasciatus strains [37, 38]. Even after injection of a high dose of ZIKV into the mosquito thorax, thus bypassing the midgut barrier, viral replication was reported to be poor in Culex mosquitoes and no viral particles were isolated from saliva [32]. The underlying reasons for these blocks are not clear but could be related to failure of ZIKV to enter host cells, replicate, disseminate etc. as well as mosquito immune responses. Among mosquito antiviral responses, small RNA-based RNA interference (RNAi) pathways are potent...
inhibitors of replication. Arbovirus replication induces the production of small RNAs: (a) viral small-interfering RNAs (vsiRNAs) that are 21 nucleotides (nt) in length and produced by an antiviral exogeneous small-interfering RNA (exo-siRNA) pathway, and (b) viral PIWI-interacting RNAs (vpiRNAs) that are 27–32 nt in length with a specific molecular signature [in sense polarity, bias for adenine at position 10 (A1); in antisense polarity U as the first nucleotide (U1)] or vpiRNA-like small RNAs missing this signature. The name PIWI derives from 'P-element Induced Wimpy testis' in Drosophila melanogaster. The exo-siRNA pathway is triggered by viral double-stranded RNA generated during the replication, and presence of 21 nt vsiRNAs is considered a key indicator of pathway induction following replication; in contrast, the origin of vpiRNAs/vpiRNA-like small RNAs and their antiviral role are less clear. vpiRNAs are produced in a Dicer 2-independent manner, and the key proteins in the pathway are PIWI family proteins Argonaute 3, Piwi5 and Piwi6. Virus-derived small RNAs usually map across the viral genome and antigenome. Moreover, non-infection-related cellular small RNAs such as endogenous siRNAs (21 nt in length) and microRNAs (usually around 22 nt in length) are an important fraction of the RNA pool within the cell [39–41].

Use of the endocellular bacteria Wolbachia [type species Wolbachia pipiens (wPip)] has been proposed as an innovative strategy for mosquito-based biocontrol of arbovirus transmission [42]. Successful transinfections of Wolbachia strains from Drosophila flies to Aedes mosquitoes have resulted in the generation of mosquito lines refractory to arboviruses including ZIKV [43–45]. Different mechanisms have been suggested to explain the molecular basis of the pathogen-blocking phenotype: upregulation of immune genes, or production of reactive oxygen species, or competition for limited resources such as cholesterol [46]. Cx. pipiens and Cx. quinquefasciatus are naturally infected with Wolbachia pipiens (named wPip), capable of manipulating host reproduction to enhance their own transmission through a phenomenon called cytoplasmic incompatibility [47]. The presence of Wolbachia could thus be an important factor affecting the permissiveness of Culex mosquitoes to ZIKV as it is for Ae. aegypti [48–50].

To assess the potential mechanism(s) underlying the blocking of ZIKV in Culex mosquitoes, we evaluated the ability of ZIKV to orally infect two lines of Cx. quinquefasciatus mosquitoes containing or free of wPip: (1) Cx. quinquefasciatus S-LAB naturally infected with wPip [51] and (2) Cx. quinquefasciatus S-LAB cleared of wPip following tetracycline (TC) treatment. Both lines S-LAB and S-LAB-TC were found to be susceptible to organophosphorus insecticides [52]. Mosquitoes were reared and maintained in controlled laboratory conditions. Before experiments, pools of 200 second-instar larvae from the two mosquito lines were homogenized and tested for wPip infection by PCR using the ankyrin domain ank2 gene (primers: F, CTTCTTCTTG AGTGTACGT and R2, TCCATATCGATCTACTCGC T) according to Attyame et al. [53]. Seven-day-old females were fed with the ZIKV strain (NC-2014-5132) isolated from a patient in April 2014 in New Caledonia [16] provided at a titre of 10^7 TCID_{50} ml^{-1} in a blood meal, in capsules of the Hemotek system maintained at 37 °C. Fully engorged females were transferred to small boxes and fed with 10% sucrose until examination. Two control groups of each mosquito line were also tested: (a) non-infected mosquitoes fed with washed rabbit erythrocytes and (b) non-infected and unfed mosquitoes only exposed to 10% sucrose. Groups of 30 females were examined at 7 and 14 days post-infection (p.i.) to estimate infection, disseminated infection and transmission rates as previously described [16]. Briefly, each mosquito was processed as follows: abdomens and thorax were examined to estimate infection, heads were examined for dissemination, and saliva was collected to estimate transmission as described [54]. Titrations were performed on Vero cells. The presence of viral particles was confirmed by cytopathic effect observation. Our results showed that all Cx. quinquefasciatus lines challenged with ZIKV were refractory to the virus whether they contained Wolbachia or not. No infection or dissemination or transmission was detected in any of the mosquito lines at 7 and 14 days p.i.

For small RNA analysis, mosquitoes were fed with rabbit blood containing ZIKV and compared with controls fed with virus-free blood or sucrose solution. RNA was isolated 3 or 7 days p.i. from groups of 10 individuals. Two replicates were produced per condition, and in total there were 24 small RNA sequencing libraries (see Table S1, available in the online Supplementary Material). Small RNAs of 15–40 nt in length were sequenced on an Illumina Hiseq 4000 at BGI Genomics. Sequence reads were mapped to the reference genome of ZIKV PE243 (GenBank accession: KX197192.1). Reads aligning to the reference genome with zero or one mismatch and alignment length from 18 to 35 bp were selected for further analysis. Based on the orientation of alignment, mapped reads were categorized into two groups, mapping to the genome and antigenome. They were further aggregated according to length, and we plotted their distribution. We did not observe any difference in ZIKV-specific small RNAs between samples obtained from ZIKV-infected or mock-infected mosquitoes (Fig. 1). With the exception of a few isolated spots, no 21 nt ZIKV-specific vsiRNAs mapping across the ZIKV genome were detected at 3 and 7 days p.i. These few spots are most likely false positives, as they occurred in both mock-infected and virus-infected mosquitoes. This can be due to sequencing errors, alignment algorithm or stochastics: the larger the vector and virus genome are, the higher the likelihood of finding matching sequences. Overall these findings indicated that ZIKV does not replicate to detectable levels in mosquito cells following a blood meal, in line with the infectivity data described above. It is worth noting that the lack of virus-derived siRNAs is not due to a general impairment of the pathway since we were able to identify endogenous siRNAs in these same samples (Fig. 2). Thus our results strongly
suggest that these Culex mosquitoes were devoid of actively replicating exogenous viruses and do not induce RNAi-based antiviral responses.

To assess the presence of other viruses, metagenomic analysis of small RNA libraries was performed [55]. Briefly, small RNA libraries were submitted to quality control, adaptors were removed and the libraries filtered to remove reads containing ambiguous nucleotides. Small RNAs mapping to the genome reference of Cx. quinquefasciatus (version CpipJ2) were removed. Remaining reads greater than 15 nt were used to assemble longer contiguous sequences. Contigs larger than 50 nt were characterized by sequence similarity searches against GenBank followed by analysis of the size profile of small RNAs. Our analysis was performed with all 24 libraries (described above) obtained from Culex mosquitoes [55]. In total, 46,039 contigs that did not map to the Culex genome were obtained from the 24 libraries (Table S1). The large majority of contigs did not show any similarity to known sequences available in GenBank. The largest contigs corresponded to the rabbit beta-globin gene and rabbit ribosomal RNA which are likely derived from the blood used for mosquito blood-feeding. A number of contigs matched retrotanspon sequences that are probably not present in the current version of the Culex genome. Regarding potential viral sequences, we observed that 66 of the 46,039 contigs showed significant sequence similarity to viruses. These 66 contigs showed similarity to one of three
viruses previously described in mosquitoes: Phasi Charoen-like virus (PCLV), Imjin River virus 1 (IRV1) and Wuhan Mosquito Virus 8 (WMV) [55–57]. Specifically, nine contigs found in nine independent libraries showed similarity to segment N, encoding nucleocapsid protein of PCLV. Another 14 contigs in 13 libraries presented similarity to the nucleoprotein of IRV1 and 43 contigs derived from 23 libraries were similar to the nucleoprotein of WMV. In all

Fig. 2. Endogenous siRNA-generating loci in *Cx. quinquefasciatus* mosquitoes. The left panel shows the size distribution of small RNAs originating from siRNA clusters identified in mosquitoes at 3 (a) and 7 days (b) post-feeding with mock- or ZIKV-infected blood. The right panel shows the size distribution of small RNAs derived from siRNA clusters normalized by Z-score considering each strand separately. 5' Base preferences of small RNAs are indicated by colour. RPM, reads per million.
cases, these potential viral contigs corresponded to the same tiny region of the virus reference. Contigs showing similarity to PCLV, IRV1 and WMV viral genomes had sizes ranging from 51 to 199 nt (Fig. 3a–c). For example, contigs homologous to PCLV corresponded to a tiny region of the S segment of this virus (Fig. 3d). The size profile and base enrichment of small RNAs derived from these potential viral contigs was consistent with production of piRNAs that showed base enrichment for U at the 5’ end with the exception of the PCLV-like sequence (Fig. S1). These small RNAs were derived almost exclusively from the antisense strand in all three cases and there was no evidence for the ping-pong amplification cycle required for the generation of secondary piRNAs (Figs 3 and S1) [41]. Notably, these three virus-like sequences represented partial ORFs and lacked any small RNAs with characteristics of siRNAs. These features are often associated with integrated viral sequences present in the host genome referred to as endogenous viral elements (EVEs) [58–60].

The role of *Culex* spp. mosquitoes in the transmission of ZIKV is still highly disputed. Here we showed that ZIKV infection does not occur in Wolbachia-infected nor in Wolbachia-free *Culex quinquefasciatus*, and subsequently neither dissemination nor transmission were detectable. Indeed, we did not detect any viral replication signatures associated with RNAi pathway induction (such as 21 nt vslRNAs) in mosquitoes fed with blood containing ZIKV. Based on the analysis of the *Cx. quinquefasciatus* small RNA libraries generated in the course of this project, we identified three separate sets of viral contigs. These sets represent the same sequence in different libraries and cover a tiny region of the reference genome that is unlikely to be a functional ORF. In addition, these viral sequences generate piRNAs but not siRNAs. Together, these results suggest that the contigs we identified correspond to EVEs and not exogenous viruses such as ZIKV. In summary, we were not able to find any sequences corresponding to exogenous viruses in the mosquitoes we analysed here. More than 20 laboratory colonies and first generations of field-collected *Culex* mosquitoes from the *Cx. pipiens* complex originated from all continents challenged with ZIKV virus isolates from all lineages failed to show competence for this virus [17, 18, 20, 28].

**Fig. 3.** Small RNA size profiles of endogenous viral elements (EVEs) found in *Cx. quinquefasciatus* mosquitoes. (a–c) Size distribution of small RNAs originating from EVEs that showed similarity to PCLV (a), IRV1 (b) and WMV (c). (d) Density of small RNAs aligned to the S segment of PCLV. E-value and contig size are shown. 5’ Base preferences of small RNAs are indicated by colour.
Although mosquitoes of the Cx. pipiens complex are known spreaders of infectious agents including arboviruses, the flavivirus St Louis encephalitis virus or the alphavirus Sindbis virus [27, 28], we suggest that this is not the case for ZIKV which belongs to the Spondweni serogroup with Spondweni virus [27, 28], we suggest that this is not the case for ZIKV strains isolated from patients in the northeast region of Brazil as performed by Guedes et al. [38], Fernandes et al. [61] showed that Cx. quinquefasciatus populations were not competent for transmitting the virus. Our data combined with numerous laboratory and field studies entirely refute the hypothesis that domestic Culex mosquitoes such as Cx. quinquefasciatus are either experimental or natural vectors of ZIKV. Whether this is due to host factors such as receptors or replication-associated factors that may differ between aedine and culicine species remains to be investigated.

Acknowledgements
The authors thank Myrielle Dupont-Rouzyrol for providing the ZIKV strain and Mylène Weill for the Culex quinquefasciatus S-LAB and S-LAB-TC lines.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The Instituto Pasteur animal facility has received accreditation from the French Ministry of Agriculture to perform experiments on live animals in compliance with the French and European regulations on care and protection of laboratory animals. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the Instituto Pasteur. This study does not involve endangered or protected species.

References
1. Boeuf P, Drummer HE, Richards JS, Scouller MJ, Beeson JG. The global threat of Zika virus to pregnancy: epidemiology, clinical perspectives, mechanisms, and impact. BMC Med 2016;14:112.
2. Lessler J, Chaisson LH, Kucirka LM, Bi Q, Grantz K et al. Assessing the global threat from Zika virus. Science 2016;353:aaf8160.
3. Wikar N, Smith DR. Zika virus: history of a newly emerging arbovirus. Lancet Infect Dis 2016;16:e119-e126.
4. Gatherer D, Kohl A. Zika virus: a previously slow pandemic spreads rapidly through the Americas. J Gen Virol 2016;97:269–273.
5. Melo AS, Aguiar RS, Amorim MM, Arruda MB, Melo FO et al. Congenital Zika virus infection: beyond neonatal microcephaly. J Am Med Assoc Neurol 2016;73:1407–1416.
6. Demir T, Kiici S. Zika virus: a new arboviral public health problem. Folia Microbiol 2016;61:523–527.
7. Espósito S, Longo MR. Guillain-Barré syndrome. Autoimmun Rev 2017;16:96–101.
8. Possas C, Brasil P, Marzoichi MC, Tanuri A, Martins RM et al. Zika puzzle in Brazil: peculiar conditions of viral introduction and dissemination – a review. Mem Inst Oswaldo Cruz 2017;112:319–327.
9. Rather IA, Kumar S, Bajpai VK, Lim J, Park YH. Prevention and control strategies to counter Zika epidemic. Front Microbiol 2017;8:305.
10. Dâlo D, Sall AA, Diagne CT, Faye O, Faye O et al. Zika virus emergence in mosquitoes in southeastern Senegal, 2011. PLoS One 2014;9:e109442.
11. Faye O, Freire CC, Lamarino A, Faye O, de Oliveira JV et al. Molecular evolution of Zika virus during its emergence in the 20th century. PLoS Negl Trop Dis 2014;8:e2636.
12. Ferreira-de-Brito A, Ribeiro IP, Miranda RM, Fernandes RS, Campos SS et al. First detection of natural infection of Aedes aegypti with Zika virus in Brazil and throughout South America. Mem Inst Oswaldo Cruz 2016;111:655–658.
13. Guerbois M, Fernandez-Salas I, Azar SR, Danis-Lozano R, Alpuche-Arandu CM et al. Outbreak of Zika virus infection, Chiapas state, Mexico, 2015, and first confirmed transmission by Aedes aegypti mosquitoes in the Americas. J Infect Dis 2016;214:1349–1356.
14. Althouse BM, Vasilakis N, Sall AA, Dâlo M, Weaver SC et al. Potential for Zika virus to establish a sylvatic transmission cycle in the Americas. PLoS Negl Trop Dis 2016;10:e0005095.
15. Gardner L, Chen N, Sarkar S. Vector status of Aedes species determines geographical risk of autochthonous Zika virus establishment. PLoS Negl Trop Dis 2017;11:e0005487.
16. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R et al. Differential susceptibilities of Aedes aegypti and Aedes albopictus from the Americas to Zika virus. PLoS Negl Trop Dis 2016;10:e004543.
17. Fernandes RS, Campos SS, Ferreira-de-Brito A, Miranda RM, Barbosa da Silva KA et al. Culex quinquefasciatus from Rio de Janeiro is not competent to transmit the local Zika virus. PLoS Negl Trop Dis 2016;10:e004993.
18. Ciota AT, Bialosuknia SM, Zink SD, Brecher M, Ehrbar DJ et al. Effects of Zika virus strain and Aedes mosquito species on vector competence. Emerg Infect Dis 2017;23:1110–1117.
19. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z et al. Competence of Aedes aegypti, Ae. albopictus, and Culex quinquefasciatus mosquitoes as Zika virus vectors, China. Emerg Infect Dis 2017;23:1085–1091.
20. Weger-Lucarelli J, Rückert C, Chotiwan N, Nguyen C, Garcia Luna SM et al. Vector competence of American mosquitoes for three strains of Zika virus. PLoS Negl Trop Dis 2016;10:e0005101.
21. Roundy CM, Azar SR, Rossi SL, Huang JH, Leal G et al. Variation in Aedes aegypti mosquito competence for Zika virus transmission. Emerg Infect Dis 2017;23:625–632.
22. Richard V, Paaafaite T, Cao-Lormeau VM. Vector competence of French polynesian Aedes aegypti and Aedes polynesiensis for Zika virus. PLoS Negl Trop Dis 2016;10:e0005024.
23. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis 2013;7:e2348.
24. Dutra HL, Rocha MN, Dias FB, Bansur SB, Caragata EP et al. Wolbachia blocks currently circulating Zika virus isolates in Brazilian Aedes aegypti mosquitoes. Cell Host Microbe 2016;19: 771–774.

25. Boccolini D, Toma L, Di Luca M, Severini F, Romi R et al. Experimental investigation of the susceptibility of Italian Culex pipiens mosquitoes to Zika virus infection. Euro Surveill 2016;21:1–3.

26. Lourenço-de-Oliveira R, Failloëx AB. Lessons learned on Zika virus vectors. PLoS Negl Trop Dis 2017;11:e0005511.

27. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. “Bird biting” mosquitoes and human disease: a review of the role of Culex pippins complex mosquitoes in epidemiology. Infect Genet Evol 2011:11:1577–1585.

28. Turell MJ. Members of the Culex pipiens complex as vectors of viruses. J Am Mosq Control Assoc 2012;28:123–126.

29. Heitmann A, Jansen S, Lühen K, Leggewie M, Badusche M et al. Experimental transmission of Zika virus by mosquitoes from central Europe. Euro Surveill 2017;22:pii: 30437.

30. Kenney JL, Romo H, Duggal NK, Tzeng WP, Burkhalter KL et al. Transmission incompetence of Culex quinquefasciatus and Culex pipiens pipiens from North America for Zika Virus. Am J Trop Med Hyg 2017;96:1235–1240.

31. Michel C, Yordanova L, Turell MJ. Acqittal of Culex quinquefasciatus in transmitting Zika virus during the French Polynesian outbreak. Acta Trop 2017;173:200–201.

32. Amraoui F, Atyame-Nten C, Vega-Rúa A, Lourenço-de-Oliveira R, Vazeille M et al. Culex mosquitoes are experimentally unable to transmit Zika virus. Euro Surveill 2016;21:1–3.

33. Hart CE, Roundy CM, Azar SR, Huang JH, Yun R et al. Zika virus vector competency of mosquitoes, Gulf Coast, United States. Emerg Infect Dis 2017;23:559–560.

34. Dodson BL, Ragson JL. Vector competency of Anopheles and Culex mosquitoes for Zika virus. PeerJ 2017;5:e3096.

35. Hall-Mendelin S, Pyke AT, Moore PR, Mackay IM, McMahon JL et al. Assessment of local mosquito species incriminates Aedes aegypti as the potential vector of Zika virus in Australia. PLoS Negl Trop Dis 2016;10:e0004959.

36. Aliota MT, Peinado SA, Osorio JE, Bartholomay LC. Acqittal of Culex quinquefasciatus and Aedes triseriatus mosquito susceptibility to Zika virus. Emerg Infect Dis 2016;22:1857–1859.

37. Guo XX, Li CX, Deng YQ, Xing D, Liu QM et al. Culex pipiens quinquefasciatus: a potential vector to transmit Zika virus. Emerg Microbes Infect 2016;5:e102.

38. Guedes DR, Paiva MH, Donato MM, Barbosa PP, Krokovsky L et al. Zika virus replication in the mosquito Culex quinquefasciatus in Brazil. Emerg Infect Microbes Infect 2017;6:e69.

39. Olson KE, Blair CD. Arbovirus-mosquito interactions: RNAi pathways. Curr Opin Virol 2015;15:119–126.

40. Blair CD, Olson KE. The role of RNA interference (RNAi) in arbovirus-vector interactions. Viruses 2015;7:820–843.

41. Donald CL, Kohl A, Schnettler E. New insights into control of arbovirus replication and spread by insect RNA interference pathways. Insects 2013;23:511–531.

42. Huang YS, Higgs S, Vanlindingham DL. Biological control strategies for mosquito vectors of arboviruses. Insects 2017;8:21.

43. Benelli G, Jeffries CL, Walker T. Biological control of mosquito vectors: past, present, and future. Insects 2016;7:52.

44. Caragata EP, Dutra HL, Moreira LA. Exploiting intimate relationships: controlling mosquito-transmitted disease with Wolbachia. Trends Parasitol 2016;32:207–218.

45. Caragata EP, Dutra HL, O’Neill SL, Moreira LA. Zika control through the bacterium Wolbachia pipientis. Future Microbiol 2016;11:1499–1502.

46. Sinkins SP. Wolbachia and arbovirus inhibition in mosquitoes. Future Microbiol 2013;8:1249–1256.

47. Serbus LR, Casper-Lindley C, Landmann F, Sullivan W. The genetics and cell biology of Wolbachia-host interactions. Annu Rev Genet 2008;42:683–707.

48. Tan CH, Wong PJ, Li Mi, Yang H, Ng LC et al. wMel limits Zika and chikungunya virus infection in a Singapore Wolbachia-introgressed Aedes aegypti strain, wMel-Sg. PLoS Negl Trop Dis 2017;11:e0005496.

49. Caragata EP, Dutra HL, Moreira LA. Inhibition of Zika virus by Wolbachia in Aedes aegypti. Microb Cell 2016;3:293–295.

50. Aliota MT, Peinado SA, Velez ID, Osorio JE. The wMel strain of Wolbachia reduces transmission of Zika virus by Aedes aegypti. Sci Rep 2016;6:28792.

51. Yan JH, Barr AR. The etiologic agent of cytoplasmic incompatibility in Culex pipiens. J Invertebr Pathol 1973;22:242–250.

52. Georgiou GP, Meltcafl RL, Gidden FE. Carbamate-resistance in mosquitoes. Selection of Culex pipiens fatigans Wiedemann (C. quinquefasciatus Say) for resistance to Baygon. Bull World Health Organ 1966;35:691–708.

53. Atyame CM, Selsuc F, Pasteur N, Weil M, Duron O. Diversification of Wolbachia endosymbiont in the Culex pipiens mosquito. Mol Biol Evol 2011;28:2761–2772.

54. Dubrulle M, Mousson L, Moutailler S, Vazeille M, Failloëx AB. Chikungunya virus and Aedes mosquitoes: saliva is infectious as soon as two days after oral infection. PLoS One 2009;4:e5895.

55. Aguier ER, Olmo RP, Paro S, Ferreira FV, de Faria IU et al. Sequence-independent characterization of viruses based on the pattern of viral small RNAs produced by the host. Nucleic Acids Res 2016;44:3477–3478.

56. Hang J, Klein TA, Kim HC, Yang Y, Jima DD et al. Genome sequences of five arboviruses in field-captured mosquitoes in a unique rural environment of South Korea. Genome Announc 2016;4: e01644–15.

57. Li CX, Shi M, Tian JH, Lin XD, Kang YJ et al. Unprecedented genomic diversity of RNA viruses in arthropods reveals the ancestry of negative-sense RNA viruses. Elife 2015;4:5979.

58. Parrish NF, Fujino K, Shiromoto Y, Iwasaki YW, Ha H et al. piRNAs derived from ancient viral processed pseudogenes as transgenerational sequence-specific immune memory in mammals. RNA 2015;21:1691–1703.

59. Lequime S, Lambrechts L. Discovery of flavivirus-derived endogenous viral elements in Anopheles mosquito genomes supports the existence of Anopheles-associated insect-specific flaviviruses. Virus Evol 2017;3:vew035.

60. Suzuki Y, Frangeul L, Dickson LB, Blanc H, Verdier Y et al. Uncovering the repertoire of endogenous flaviviral elements in Aedes mosquito genomes. J Virol 2017;91:e00571-17.

61. Fernandes RS, Campos SS, Ribeiro PS, Raphael LM, Bonaldo MC et al. Culex quinquefasciatus from areas with the highest incidence of microcephaly associated with Zika virus infections in the north-east region of Brazil are refractory to the virus. Mem Inst Oswaldo Cruz 2017;112:577–579.

62. Duffy MR, Chen TH, Hancock WT, Powers AM, Kool JL et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med 2009;360:2536–2543.

63. Grard G, Caron M, Mombo IM, Nikoghe D, Mbouo Ondo S et al. Zika virus in Gabon (Central Africa)–2007: a new threat from Aedes albopictus? PLoS Negl Trop Dis 2014;8:e2681.