Chapter 9
Mosquitoes as Arbovirus Vectors: From Species Identification to Vector Competence

Claudia Schulz and Stefanie Christine Becker

Abstract Mosquitoes and other arthropods transmit a large number of medically important pathogens, in particular viruses. These arthropod-borne viruses (arboviruses) include a wide variety of RNA viruses belonging to the *Flaviviridae* family (*West Nile virus* (WNV), *Usutu virus* (USUV), *Dengue virus* (DENV), *Japanese encephalitis virus* (JEV), *Zika virus* (ZIKV)), the *Togaviridae* family (*Chikungunya virus* (CHIKV)), and *Bunyavirales* order (*Rift Valley fever virus* (RVFV)) (please refer also to Table 9.1). Arboviral transmission to humans and livestock constitutes a major threat to public health and economy as illustrated by the emergence of ZIKV in the Americas, RVFV outbreaks in Africa, and the worldwide outbreaks of DENV. To answer the question if those viral pathogens also pose a risk to Europe, we need to first answer the key questions (summarized in Fig. 9.1):

1. **Who** could contribute to such an outbreak? Information about mosquito species resident or imported, potential hosts and viruses able to infect vectors and hosts in Germany is needed.
2. **Where** would competent mosquito species meet favorable conditions for transmission? Information on the minimum requirements for efficient replication of the virus in a given vector species and subsequent transmission is needed.
3. **How** do viruses and vectors interact to facilitate transmission? Information on the vector immunity, vector physiology, vector genetics, and vector microbiomes is needed.

Keywords Zoonotic arbovirus · Europe experimental infection · Vector competence · Antiviral immune response taxonomy

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9.1 Who Could Contribute to an Arbovirus Outbreak

9.1.1 Taxonomy and Mosquito Surveillance in Europe

As the spread of mosquito-borne arboviruses is dependent on the presence of a suitable mosquito vector, the knowledge of the mosquito species distribution and vector competence of these mosquitoes belongs to the most crucial factors for estimations about the risk of mosquito-virus emergence to new areas or maintenance of (endemic) arboviruses within particular regions. The first critical issue for mosquito surveillance programs is the exact classification of species (Fig. 9.1).

To facilitate detection of different species, several methods have been proposed. Classical morphology is used as the first line of classification. Several keys for morphological discrimination have been published. The morphological characteristics described by Mohrig (1969) and Becker et al. (2010) have been most commonly used for species identification in surveillance programs in Germany. These programs include several university- and organization-driven approaches, some as a part of the European project VBORNET (http://www.vbornet.eu/) or the citizen science project "Mückenatlas" (Walther and Kampen 2017). All those projects have made large progress in redefining the mosquito fauna in Europe and Germany. Especially the "Mückenatlas" project has also proven a very sensitive tool to detect new and invasive species as, for example, several new populations of *Aedes japonicus japonicus* in North Rhine-Westphalia and Lower Saxony and *Aedes albopictus* populations in Baden-Wuerttemberg (Kampen et al. 2016a; Werner and Kampen 2013; Werner et al. 2012; Zielke et al. 2014).

Within all programs, the classical morphology has proven a useful tool. However, the accuracy of classical morphological classification is strongly dependent on expert knowledge and the availability of good-quality mosquito specimens. Furthermore, several cryptic species allow only for classification according to male mosquitoes, which are often not attracted by the traps used for surveillance programs. Especially females of the *Culex pipiens* complex (Fonseca et al. 2004) and the *Anopheles maculipennis* complex (Kronefeld et al. 2012, 2014; Proft et al. 1999) turned out to be difficult or impossible to distinguish in case of morphologically similar sibling species, such as *Culex torrentium* and the two *Culex pipiens pipiens* biotypes *pipiens* and *molestus* or mosquito species belonging to the *Anopheles messeae/daciae* complex. Both species complexes are of major importance for disease transmission: *Culex pipiens* a main vector for WNV, USUV, or RVFV and *Anopheles maculipennis* as a potential vector for *Plasmodium* parasites. Hence, classification methods besides morphology are needed to reach a satisfactory level of species discrimination (Bickford et al. 2007).

The use of morphometric analysis as a qualitative tool for species discrimination has expanded during the past years (reviewed by Lorenz et al. (2017)). In particular wing shape has been used for morphometric comparison in mosquito studies. Wilke et al. (2016) have established a protocol for geometric wing morphometries to identify a broad range of medically important mosquito species belonging to the *Aedes*, *Culex*, and *Anopheles* genera. To do so, 18 landmarks at wing vein intersections
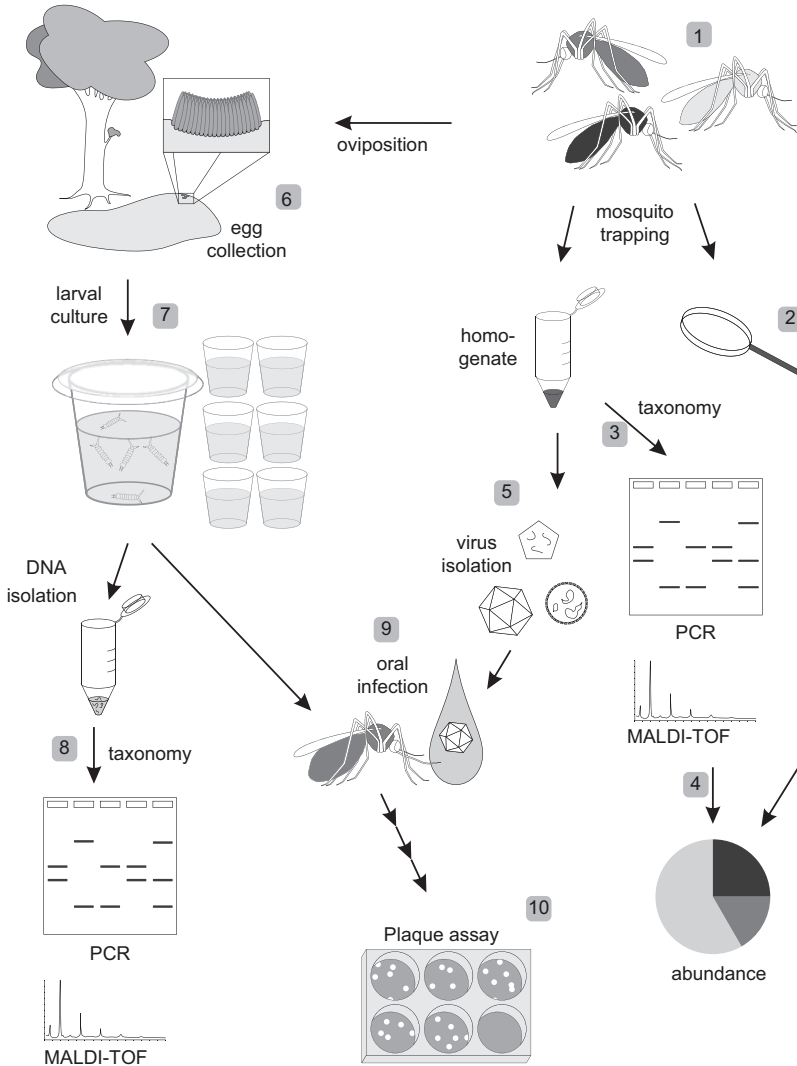
Fig. 9.1 Graphical representation of vector competence assay. The analysis of resident mosquito populations for virus presence and vector competence for the respective virus starts with the collection of mosquitoes (1). Subsequently, the mosquitoes are subjected to morphological taxonomic classification (2) and are pooled according to species and location. Mosquito pools are homogenized to isolate nucleic acids for PCR and proteins for MALD-TOF MS. These data are used for taxonomic confirmation (3) and abundance statistics (4) or virus screening. Virus-positive pools will be used for virus isolation (5) which can then be used for vector competence assays via oral infection (9). To obtain mosquito samples for vector competence assays, eggs of resident mosquito populations are collected (6) and reared in the laboratory (7). From each larval culture, some specimens will be used for taxonomic identification (8). Larvae from the same location and species are pooled, and emerging adult females will be used for vector competence assays. New virus isolates are mixed with blood and fed to 4–7-day-old female mosquitoes (9). After different times of infection, some mosquitoes are sacrificed, and body infection rates (IR), dissemination rates (DR), and transmission rates (TR) will be measured by virus titration (10)

were collected from digitalized photographs of female wings. Mosquito genera were classified with 99% accuracy and species even with 100% accuracy, demonstrating the power of the approach (Wilke et al. 2016).

Several other groups also used this method to discriminate female samples of closely related cryptic species. Lorenz et al. (2012) analyzed the same 18 landmarks to distinguish between *Anopheles cruzi, Anopheles homunculus*, and *Anopheles bellator* mosquitoes and reached 78–88% accuracy. For the *Culex* complex, differences in wing venation were already described by Natvig (1948) and Mohrig (1969), who also proposed to use these differences for species discrimination. Especially the vein R2/3 was found informative for differentiation of *Culex pipiens* and *Culex torrentium* females. Borstler et al. (2014) used general wing morphology and the R2/3 indices for discrimination of *Culex pipiens* and *Culex torrentium* collected in Germany. Their study revealed more than 91% accuracy in the multivariant morphometric analysis using several wing landmarks and 90% correct species identification when only using the R2/3 vein indices. Thus, the morphometric discrimination method has been proven to be a stable and reliable method with success rates of 70–100% for correct reclassification (Lorenz et al. 2017). It is particularly tempting that this morphometric method has been shown to be most accurate in female mosquitoes, the main object of interest in the context of vector-borne diseases.

Although geometric morphometry is a quick and easy to use method, it should be noted that data capturing and identification of landmarks are still a critical issue. Furthermore, in large-scale surveillance programs, a certain degree of automatization of landmark detection and automatic species identification needs to be made, in order to ensure a timely species identification (Lorenz et al. 2017). Thus, molecular methods for large-scale species identification are still needed. In recent years, several advances in the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have been explored to achieve species differentiation. MALDI-TOF MS has been extensively used in bacterial diagnostics (Dierig et al. 2015) and for species identification of *Drosophila* (Feltens et al. 2010) as well as of relevant vector species such as *Culicoides* biting midges (Kaufmann et al. 2012), *Phlebotomus* sand flies (Mathis et al. 2015), and *Ixodes* ticks (Yssouf et al. 2013a, 2015). Due to the extensive use in diagnostics, a lot of laboratories adjacent to clinics have already implemented MALDI-TOF MS facilities that can easily be used for mosquito surveillance programs. The adaptation of the MALDI-TOF MS for mosquito species identification has made great advances in the past years. Yssouf et al. (2013b) described this technique to analyze samples from tropical areas and were able to establish profiles from 20 mosquito species collected in La Réunion Island and Senegal. In this study, a reliable classification on subspecies level was achieved as demonstrated for the M and S forms of *Anopheles gambiae.* In total, 100% of the samples were identified correctly after generation of a spectra database. Therefore, this score was set a cutoff value for species identification. However, the method was not considered suitable for mosquito phylogeny yet. To refine the database and to add new species to the collection, Yssouf et al. (2014) conducted a subsequent study using mosquito samples of 11 different species collected at different sites in France and Sweden. After the generation of reference

samples based on previous morphological characterization (Becker et al. 2010), 88.5% of the samples were identified correctly. These and other studies (Raharimalala et al. 2017; Schaffner et al. 2014) showed the feasibility and reliability of MALDI-TOF MS for mosquito species identification. Furthermore, the method is usually described as an inexpensive and easily implementable approach. However, a certain degree of instability was recently detected in mosquito samples collected in different countries, highlighting the importance to establish an international database to assure correct mosquito species identification.

Another very sensitive and reliable method for species identification and the differentiation of cryptic species or biotypes is their genetic characterization by conventional and real-time PCR using phylogenetic markers: either chromosomal markers such as the acetylcholinesterase 2 (*ace2*) gene, the second internal transcribed spacer (ITS2), and the microsatellite locus *CQ11* or mitochondrial barcoding based on the cytochrome oxidase I (*COI*) gene. The use of the *ace2* locus as a diagnostic criterion for the differentiation of *Culex pipiens* complex (*Culex pipiens*, *Culex quinquefasciatus*, *Culex p. pallens*, *Culex australicus*), *Culex torrentium*, and *Culex pervigilans* mosquitoes was developed by Smith and Fonseca (2004). Mosquitoes of these species were collected across the world and subjected to PCR analysis using diagnostic primers located between exons 2 and 3 of the *ace2* genetic locus. The *ace2* PCR assay was able to distinguish and to detect hybridization events between the mentioned *Culex* species, for example, hybridization of *Culex quinquefasciatus* and *Culex pipiens*, but the two bioforms *Culex pipiens pipiens* biotype *pipiens* and *Culex pipiens pipiens* biotype *molestus* could not be discriminated. The two biotypes show different feeding patterns and (breeding) habitat preferences with *Culex pipiens pipiens* biotype *molestus* being more anthropophilic and adapted to urban habitats, whereas the biotype *pipiens* is more ornithophilic and adapted to a wide range of natural habitats. These differences in lifestyle can have major impact on their ability to act as vectors for viruses such as WNV. Indeed, *Culex pipiens pipiens* biotype hybrids have been widely discussed as potential bridge vectors between birds and humans for bird-associated viruses such as WNV. Thus, the correct identification of these two biotypes can be crucial for correct risk assessment. To improve biotype differentiation, Bahnck and Fonseca sequenced microsatellite loci of the *Culex pipiens* complex and found that the *CQ11* locus was suitable for the diagnosis and differentiation of two *Culex pipiens* biotypes (Bahnck and Fonseca 2006).

The two assays *ace2* and *CQ11* (Bahnck and Fonseca 2006; Smith and Fonseca 2004) were used to design a multiplex qPCR assay which allows the differentiation of *Culex* species, biotypes, and biotype hybrids within one reaction (Rudolf et al. 2013). Using a large collection of about 349 morphologically well-defined mosquito specimens (consisting of 227 *Culex pipiens* biotype *pipiens*, 3 *Culex pipiens* biotype *molestus*, and 119 *Culex torrentium* samples), the assay was evaluated and revealed 100% specificity for the respective *Culex* species or biotypes (Rudolf et al. 2013). The analysis of 16,566 *Culex* samples collected at different trapping sites in Germany with this multiplex qPCR revealed that *Culex pipiens* biotype hybrids are also present in Germany. Furthermore, the expansion of *Culex torrentium* in Central

Europe was confirmed with more than 50% of the collected specimens containing *Culex torrentium* at some sample locations in Germany. The same multiplex qPCR method was also used for a surveillance study in the Emilia-Romagna in Italy, which revealed that all (100%) of the 24,165 tested mosquitoes were *Culex pipiens* and that *Culex torrentium* was absent at these sample locations (Calzolari et al. 2016). This is in agreement with other studies performed across Europe by Hesson et al. (2014) analyzing 2559 larval samples from 138 collection sites in 13 European countries. This study found *Culex torrentium* more prevalent than *Culex pipiens* in Central and Northern Europe but mostly absent in Southern Europe. The study by Hesson et al. (2014) used a different method based on the amplification of the 3′-end of the *COI* locus, subsequent restriction digest, and sequencing (Hesson et al. 2010) for genetic characterization of *Culex pipiens* and *Culex torrentium*. The mitochondrial *COI* gene is often used for species identification or confirmation of morphological classification. To do so, the 5′ part *COI* gene is amplified with generic primer sets, and the PCR products are usually sequenced and analyzed (Folmer et al. 1994). According to Hebert et al. (2003), this method is adequate to "barcode" most animal species with an intraspecies variation mostly below 2% and thus allows for reliable intraspecies identification. The *COI* barcoding has then been used in a large-scale approach such as the International Barcode of Life (iBOL) project creating a reference database BOLD (www.boldsystems.org). In subsequent years, the method had become a standard technique to identify mosquito species from different countries around the world including China (Wang et al. 2012), Pakistan (Ashfaq et al. 2014), Chile, and Sweden (Engdahl et al. 2014). However, in the Swedish study (Engdahl et al. 2014), some inconsistencies between morphological discrimination and barcoding results were observed. Furthermore, the method may cause inconclusive results in closely related species such as species belonging to the *Culex pipiens* complex. In this case, additional methods such as the restriction analysis of the *COI* PCR fragment described by Hesson et al. (2010) or additional PCRs for the *ace-2* and *CQ11* loci (Bahnck and Fonseca 2006; Fonseca et al. 2004; Rudolf et al. 2013) can be advantageous.

The *Anopheles maculipennis* complex comprises 10–12 Palearctic species (Harbach 2004), and members of the complex have been associated with *Plasmodium*, Sindbis virus, and Batai virus transmission in Europe (Jost et al. 2010, 2011b; Kampen et al. 2016b). In light of the risk for reintroduction of *Plasmodium* species by enhanced global travel, identification of potential malaria vectors is of major interest. Therefore, in 1999, Proft et al. (1999) developed a diagnostic PCR method for identification of the members of this complex that are otherwise indistinguishable. This PCR assay was based on the *ITS2* region, which had been previously used for differentiation of other complexes (Crabtree et al. 1995; Wesson et al. 1992). The PCR products were sequenced, results were compared with morphological classification, and a stable PCR assay for identification of *Anopheles atroparvus*, *Anopheles melanoon*, *Anopheles sacharovi*, *Anopheles maculipennis s. s.*, *Anopheles messeae*, and *Anopheles labranchiae* was established. In the following years, surveillance studies revealed that particularly *Anopheles messeae* is widespread across Central Europe. However, in a study of Novikov and Shechenko in

2001, it became evident that *Anopheles messeae* was not a single species (Novikov and Shevchenko 2001) but represents two cryptic species, *Anopheles messeae* and *Anopheles daciae*, which was confirmed 3 years later (Nicolescu et al. 2004). To differentiate these cryptic species, the *ITS2* assay was refined by the addition of a restriction fragment length polymorphism (RFLP) analysis after *ITS2* amplification (Kronefeld et al. 2012, 2014). Also, Weitzel et al. (2012) refined the *ITS2* analysis to facilitate *Anopheles messeae* and *Anopheles daciae* differentiation by adding a sequencing reaction after initial amplification. However, both protocols are somewhat laborious and prone to contamination. Thus, in 2016, Luhken et al. (2016) described a new multiplex qPCR method to discriminate between the most prominent members of the *Anopheles maculipennis* complex in Central Europe (i.e., *Anopheles maculipennis*, *Anopheles messeae s.l.*, and *Anopheles atroparvus*) and a fluorescence resonance energy transfer (FRET)-based assay to distinguish between *Anopheles messeae s.s* and *Anopheles daciae*. As a result of the large-scale study following the establishment of this method, 1445 mosquitoes from Germany were screened, and the superior spread of *Anopheles messeae* in Central Europe was confirmed with approximately 70% of the samples belonging to this species.

9.1.2   Virus Surveillance in Europe

During the last decade, multiple previously exotic arboviruses that belong to different virus families which may have considerable implications on human and/or animal health have emerged in Europe. Notable examples are mosquito-borne viruses such as CHIKV (*Togaviridae*) and DENV and ZIKV (*Flaviviridae*) as well as *Culicoides*-borne viruses such as *Bluetongue virus* serotype 8 (BTV-8; *Reoviridae*) and *Schmallenberg virus* (SBV, an *Orthobunyavirus* within the *Peribunyaviridae* family and *Bunyavirales* order). Their unexpected emergence—facilitated by globalization and climate change—highlight the risk of future introductions and spread of additional pathogenic arboviruses to Europe such as (1) mosquito-borne *Bunyamwera orthobunyavirus* (BUNV, *Peribunyaviridae*), *O'nyong-nyong virus* (ONNV, *Togaviridae*), (2) mosquito- and *Phlebotomus*-borne RVFV (*Phenuiviridae*), (3) *Culicoides*-borne *Oropouche virus* (OROV, *Peribunyaviridae*), or (4) tick-borne *Crimean-Congo Hemorrhagic fever virus* (CCHFV, *Nairoviridae*) (Amraoui and Failloux 2016; Brustolin et al. 2017; Carpenter et al. 2013; Heitmann et al. 2017; Negredo et al. 2017; Rudolf 2015; Tappe et al. 2014). Importantly, the introduction of novel viruses to regions where related (endemic) viruses circulate can result in reassortment and a consequential change in pathogenicity and phenotype (Briese et al. 2013; Rudolf 2015). A large outbreak of hemorrhagic fever in humans was reported in Africa in the 1990s caused by a reassortant of African strains of BUNV and *Batai virus* (BATV, an infraspecies of BUNV), namely, *Ngari virus* (NRIV) (Gerrard et al. 2004). Repeated introductions of emerging zoonotic mosquito-borne viruses in addition to CHIKV and ZIKV have been reported in Europe, including DENV and *Yellow fever virus* (YFV, *Flaviviridae*) (Húbalek 2008). The detection of

genome fragments of JEV, another flavivirus, in *Culex pipiens* mosquitoes caught in Italy (2010/2011) indicated a repeated introduction or enzootic circulation of JEV or of a related virus in Southern Europe (Cleton et al. 2014; de Wispelaere et al. 2017). A series of repeated disease outbreaks in humans were caused by various zoonotic mosquito-borne viruses endemic or emerging in Europe. In recent years, human virus infections or disease outbreaks were reported in Europe, including chikungunya in Italy (2007) and France (2010, 2014); dengue in Croatia (2010), France (2010, 2013, 2014), and Portugal (Madeira; 2012); usutu in Italy (2009) and Croatia (2013) (Kampen and Werner 2015); and West Nile fever in Austria (2009, 2010, 2014–2016), Croatia (2012–2013), France (2015), Greece (2010–2014), and Italy (2010–2015) (ages 2017; Gossner et al. 2017; Kampen and Werner 2015). WNV is considered endemic in Europe. However, there are several neglected zoonotic arboviruses circulating in Europe that may (at least occasionally) cause disease in humans and animals: BATV, *Tahyna virus* (TAHV, infraspecies of *California encephalitis orthobunyavirus*, *Peribunyaviridae*), SINV (*Alphavirus, Togaviridae*), and *Inkoo virus* (INKV, infraspecies of *California encephalitis orthobunyavirus*) (Eckerle et al. 2018; Húbalek 2008). In general, three groups of mosquito-borne viruses can be distinguished according to their clinical signs: (1) fever-arthralgia-rash (e.g., DENV, CHIKV, ONNV, ZIKV, WNV), (2) affection of the central nervous system (e.g., DENV, ZIKV, WNV), and (3) hemorrhagic fever (e.g., DENV, RVFV) (reviewed by Eckerle et al. (2018); Húbalek (2008); Kampen and Werner (2015)).

9.2 Where Would Competent Mosquito Species Meet Favorable Conditions for Transmission?

9.2.1 Factors for Arbovirus Transmission

Implications for public health emitting from arboviruses depend on key factors that influence vectorial capacity such as barriers to the infection and transmission of arboviruses to mosquitoes (Hardy et al. 1983; Mellor 2009) as well as biotic and abiotic factors that vice versa have an effect on the intrinsic infection barriers. Climate, in particular temperature and precipitation, and ecological factors (in particular land use, anthropogenic disturbance/urbanization) are main abiotic drivers determining the probability of transmission within a given region (Húbalek 2008; Junglen 2016; Kramer 2016). Biotic factors include (1) the susceptibility to infection (e.g., immunogenetics), host diversity, density, behavior, and seasonal abundance of vertebrate hosts (e.g., migration of birds) and their capability to efficiently amplify and transmit the virus to mosquitoes; (2) the vectorial capacity, density, and (opportunistic) feeding preferences concerning the blood source of invertebrate hosts; (3) the genotype and phenotype/pathogenicity of a virus; as well as (4) the interaction, variability, and adaptation between virus genotype x vector genotype and immune system x vertebrate genetics and immune system (Fros et al. 2015a; Kramer 2016; Lambrechts et al. 2009b).

9.2.2 Barriers to the Infection and Transmission of Arboviruses by Mosquito Vectors

Vector competence generally depends on intrinsic factors of the mosquito (Hardy et al. 1983) (Fig. 9.2). After the ingestion of an infectious blood meal, the pathogen has to overcome several barriers in the insect host before transmission with injected saliva during a blood meal on a vertebrate host can occur. The midgut infection barrier (MIB) and midgut escape barrier (MEB) may impede virus passing the midgut cells into the hemocoel (Hardy et al. 1983; Mellor 2009; Mellor et al. 2000). An interference of virus dissemination from the fat cells (dissemination barrier, DB) in the hemocoel is explained by the fact that the fat body plays a role in the insect immune response and prevents infection of other tissues (see section innate immunity below (Mellor 2009)). Further barriers include the salivary gland infection
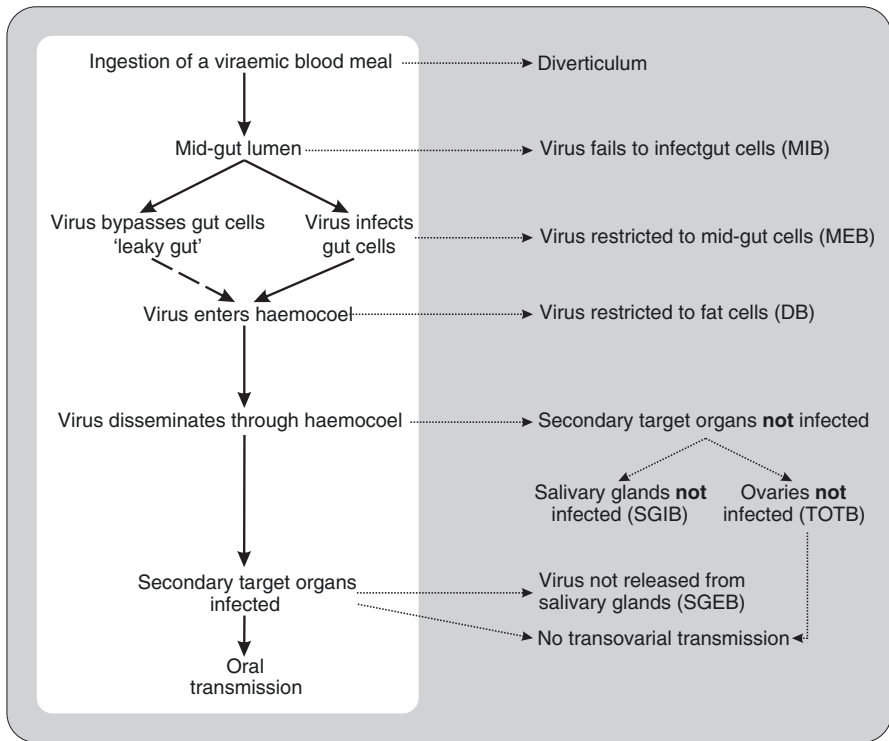

Fig. 9.2 Intrinsic barriers to infection and transmission of arboviruses in mosquitoes. The midgut infection barrier (MIB), midgut escape barrier (MEB), dissemination barrier (DB), salivary gland infection barrier (SGIB), salivary gland escape barrier (SGEB), and transovarial transmission barrier (TOTB) are potentially interfering with infection, dissemination, and transmission of viruses after the ingestion of an infectious blood meal by a mosquito. Only virus release in the saliva and transmission by bite of a vertebrate host confirm the completion of the extrinsic incubation period (EIP) and vector competence of a mosquito (Adapted from Mellor et al. 2000; Hardy et al. 1983)

barrier (SGIB), salivary gland escape barrier (SGEB), and transovarial transmission barrier (TOTB) (summarized in Fig. 9.2). Mosquito females that survive the extrinsic incubation period (EIP, the interval between ingestion of a virus and the earliest time at which virus is released in saliva) potentially remain infectious throughout their life (Hardy et al. 1983; Mellor 2009; Mellor et al. 2000). Experimental vector competence studies regularly include analysis of the infection rate (IR), dissemination rate (DR), and transmission rate (TR). Due to the various possible barriers of an insect host, the TR (defined as the number of mosquitoes with virus-positive saliva per number of virus-positive mosquito bodies (Heitmann et al. 2017)) provides the most important information about the vector competence of a mosquito since only virus transmission by saliva during an insect bite is infectious for the vertebrate host. On the other hand, differences in the IR, DR, and TR may give useful information about possible barriers for a certain virus within an insect host. Furthermore, the EIP depends on the invertebrate host-virus interaction and on the ambient temperature (Mellor 2009) (Table 9.1). Mosquitoes that are not (typical) vectors for a given virus may get competent if reared at elevated temperatures, as reported for *Culicoides nubeculosus* biting midges (a potential vector of BTV). A possible reason is that an increased temperature during the immature stage of the mosquito may compromise the integrity of the gut wall enabling virus to bypass the gut barrier ("leaky gut" phenomenon) (Wittmann and Baylis 2000). In adult mosquitoes, crucial differences in vector competence of *Aedes albopictus* for ZIKV depending on the ambient temperature have been demonstrated by Heitmann et al. (2017). German and Italian populations of *Aedes albopictus* that were infected with ZIKV and kept at 18 °C were not found competent for ZIKV transmission (TR of 0%), while a TR of 18–20% was found in *Aedes albopictus* kept at 27 °C after an EIP of 14 days. In contrast, none of the *Culex pipiens* biotype *pipiens*, *Culex pipiens* biotype *molestus*, and *Culex torrentium* populations were found competent at 18 or 27 °C. Similar results were reported for Italian and French *Aedes albopictus* populations (TR of 4–29%) and for an Italian *Culex pipiens* (TR of 0%) population kept at 26 or 28 °C (Boccolini et al. 2016; Di Luca et al. 2016; Jupille et al. 2016). The midgut barriers (MIB and MEB) may be circumvented by using intrathoracic instead of oral infection. Intrathoracically infected mosquitoes show a considerable higher IR and TR (up to 100%) than orally infected mosquitoes as demonstrated for USUV (TR of 69%, *Culex pipiens*) and WNV (TR of 22–33%, *Culex pipiens*) (Fros et al. 2015a, b). This can lead to overestimation of IR and TR and consequently to misleading interpretation of vector competence (Fros et al. 2015a, b). Fu et al. (1999) suggested that, following intrathoracical inoculation, virus levels in the hemocoel exceed the virus amount that can be cleared by fat bodies. Another important factor for efficient infection of mosquitoes is the orally ingested virus dose. Only vertebrate species that produce viremia (sufficiently high for infection) can be regarded as amplifying hosts (Húbalek 2008) as it is the case for WNV in birds, but not for WNV in horses or humans (Angenvoort et al. 2013; Bunning et al. 2002; Hayes et al. 2005). When *Culex quinquefasciatus* mosquitoes are experimentally infected with a low (10^4 plaque-forming units per mL (PFU/mL)) or a higher (10^6 PFU/mL) dose of ZIKV, only mosquitoes that ingested the higher dose got infected (Guedes et al. 2017). In

Table 9.1 Summary of experimental studies of vector competence for ZIKV, DENV, CHIKV, USUV, RVFV, JEV, and WNV of mosquito species collected in Europe (data of positive control mosquito vectors used in the studies are not shown)

Virus	Origin	Mosquito Species	Origin/no. field/lab/NA	Trial (all o.f.) Temperature (°C)/relative humidity (%)	EIP (days)	Virus detection method	TR (%)/no. of colonies	Result Vector competent (yes/no)	Reference
[a]									
CHIKV	La Réunion, strain E1A226V	Aedes albopictus	Italy/2f	28/NA	14	PCR	NA (10–100)	Y	Talbalaghi et al. (2010)
CHIKV	La Réunion, strain E1A226V	Aedes albopictus	Corsica/2f	28/NA	14	PCR	NA (80–100)	Y	Moutailler et al. (2009)
CHIKV	Strain NC/2011-568	Aedes detritus	UK/1f	21/70	17	PCR	0	N	Blagrove et al. (2016)
DENV	Thailand, serotype 2	Aedes detritus	UK/1f	21/70	17	PCR	0	N	Blagrove et al. (2016)
JEV	cDNA clones[b]: 1. Taiwan, 1985, g3 strain RP-9 or 2. China, 2009, g5 strain XZ0934	Aedes albopictus	France/11	26/80	13	FFA	20–63%	Y	De Wispelaere et al. (2017)
JEV	Malaysia, 1952, g5, Muar strain	Aedes detritus	UK/1f	23/70–90 28/70–90	7–21 14 7–21 14	Vi	13 3 25 17	Y	Mackenzie-Impoinvil et al. (2015)
JEV	Japan, 1935, g3, strain Nakayama[c]	Aedes japonicus japonicus	Germany/1f	25/85	14	PCR	100[d]	Y	Huber et al. (2014a)

(continued)

Table 9.1 (continued)

Virus	Origin	Mosquito Species	Origin/no. field/lab/NA	Trial (all o.f.) Temperature (°C)/relative humidity (%)	EIP (days)	Virus detection method	TR (%)/no. of colonies	Result Vector competent (yes/no)	Reference
[a]									
JEV	cDNA clones[b]: 1. Taiwan, 1985, g3 strain RP-9 or 2. China, 2009, g5 strain XZ0934	Culex pipiens	France/11	26/80	13	FFA	12–41%	Y	De Wispelaere et al. (2017)
RVFV	South Africa/strain RVF 56/74	Aedes albopictus	Spain/1f	Ø 22 night to Ø 26 day/80	14	Vi	NA ($n = 1$)	Y	Brustolin et al. (2017)
RVFV	Avirulent Clone 13[e]	Aedes caspius	France/1f	28/80	14	IFA	7[d]	Y	Moutailler et al. (2008)
RVFV	Virulent ZH548 avirul. Clone 13[e]	Aedes detritus	France/1f	28/80	14	IFA	13[d] 0[d]	Y	Moutailler et al. (2008)
RVFV	Virulent ZH548 avirul. Clone 13[e]	Aedes vexans	Germany/11	28/80	14	IFA	8[d] 25[d]	Y	Moutailler et al. (2008)
RVFV	Virulent ZH548 avirul. Clone 13[e]	Culex pipiens	France/1f France/2f	28/80	14	IFA	14[d] 4–9[d]	Y	Moutailler et al. (2008)
RVFV	Virulent ZH548 avirul. Clone 13[e]	Culex pipiens	Cyprus/1NA	28/80	14	IFA	30[d] 14[d]	Y	Moutailler et al. (2008)
RVFV	South Africa/strain RVF 56/74	Cx p.pipiens b. molestus	Spain/1f	Ø 22 night to Ø 26 day/80	14	Vi	0	N	Brustolin et al. (2017)

						Vi and/or PCR			
RVFV	South Africa/strain RVF 56/74	Cx p.pipiens b. pipiens x b. molestus (hybrid)	Spain/1f	Ø 22 night to Ø 26 day/80	14	Vi and/or PCR	NA (n = 6)	Y	Brustolin et al. (2017)
USUV	Italy, 2011, 3 strains[c]	Aedes albopictus	Italy/1f	28 ± 1/80	14	PCR	0	N	Puggioli et al. (2017)
USUV	USUV, Bologna/09[c]	Culex pipiens	NL/1I	28/60	14	Vi	69%	Y	Fros et al. (2015b)
WNV	France, lineage 1, Camargue 2001, Eva Ref-2651	Aedes albopictus	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	NA (1 group)	Y	Brustolin et al. (2016)
WNV	Italy, lineage 2, strain 178907/2013	Aedes albopictus	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	NA (1 group)	Y	Brustolin et al. (2016)
WNV	Sardinia, 2011, lineage 1, strain Ma V3	Aedes albopictus	Italy/1f	27 ± 1/70	14	Vi	50	Y	Fortuna et al. (2015a)
WNV	USA, lineage 1, strain NY99	Aedes japonicus japonicus	Germany/1f	25/85	14	PCR	0[d]	N	Huber et al. (2014a, b)
WNV	USA, lineage 1, strain NY-99, NCBI DQ211652	Aedes japonicus japonicus	Switzerland/1f	24 ± 7/45–90	12 to 15	Vi	NA (4 pools)	Y	Wagner et al. (2018)
WNV	Italy, lineage 1, strain Italy/2009/FIN[c]	Aedes japonicus japonicus	Switzerland/1f	24 ± 7/45–90	12 to 15	Vi	NA (1 pool)	Y	Wagner et al. (2018)

(continued)

Table 9.1 (continued)

Virus		Mosquito	Trial (all o.f.)					Result	Reference
	Origin[a]	Species	Origin/no. field/lab/NA	Temperature (°C)/relative humidity (%)	EIP (days)	Virus detection method	TR (%)/no. of colonies	Vector competent (yes/no)	
WNV	USA, lineage 1, strain NY-99	*Aedes detritus*	UK/1f	21/70	17	PCR	21	Y	Blagrove et al. (2016)
WNV	USA, lineage 1, strain NY-99[c]	*Culex pipiens*	Switzerland/1f	24 ± 7/45–90	12 to 15	Vi	NA (4 pools)	Y	Wagner et al. (2018)
WNV	Italy, lineage 1, strain Italy/2009/ FIN[c]	*Culex pipiens*	Switzerland/1f	24 ± 7/45–90	12 to 15	Vi	0	N	Wagner et al. (2018)
WNV	Sardinia, 2011, lineage 1, strain Ma V3	*Culex pipiens*	Italy/2f, 2l	28 ± 1/70	6 to 32	Vi	37 to 47	Y	Fortuna et al. (2015b)
WNV	Sardinia, 2011, lineage 1, strain Ma V3	*Culex pipiens*	Italy/1f	27 ± 1/70	14	Vi	33	Y	Fortuna et al. (2015a)
WNV	USA, lineage 1, strain NY-99	*Culex pipiens*	NL/1l	23/60	14	Vi	22	Y	Fros et al. (2015a)
WNV	Greece, lineage 2, Gr-2010	*Culex pipiens*	NL/1l	23/60	14	Vi	24	Y	Fros et al. (2015a)
WNV	Greece, lineage 2, Gr-2010[c]	*Culex pipiens*	NL/1l	28/60	14	Vi	33	Y	Fros et al. (2015b)

WNV	France, lineage 1, Camargue 2001, Eva Ref-2651	Cx p.pipiens b. molestus	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	0	N	Brustolin et al. (2016)
WNV	Italy, lineage 2, strain 178907/2013	Cx p.pipiens b. molestus	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	0	N	Brustolin et al. (2016)
WNV	France, lineage 1, Camargue 2001, Eva Ref-2651	Cx p.pipiens b. pipiens x b. molestus (hybrid)	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	0	N	Brustolin et al. (2016)
WNV	Italy, lineage 2, strain 178907/2013	Cx p.pipiens b. pipiens x Cx p.pipiens b. molestus (hybrid)	Spain/1f	Ø 21.3 night to Ø 27.7 day/70	12	Vi	NA (1 group)	Y	Brustolin et al. (2016)
ZIKV	Asian genotype?	Aedes albopictus	Italy/1NA	18/80 27/80	14 14	Vi Vi	0 18	N Y	Heitmann et al. (2017)
ZIKV	Asian genotype?	Aedes albopictus	Germany/1NA	18/80 27/80	14 14	Vi Vi	0 20	N Y	Heitmann et al. (2017)
ZIKV	Asian genotype	Aedes albopictus	France/1f	28 ± 1/80	14	Vi	4	Y	Jupille et al. (2016)
ZIKV	Asian genotype	Aedes albopictus	Italy/1f	26 ± 1/70	11 to 14	PCR	29	Y	Di Luca et al. (2016)
ZIKV	Asian genotype?	Cx p.pipiens b. pipiens	Germany/1NA	18/80 27/80	14 14	Vi Vi	0 0	N N	Heitmann et al. (2017)
ZIKV	Asian genotype?	Cx p.pipiens b. molestus	Germany/1NA	18/80 27/80	14 14	Vi Vi	0 0	N N	Heitmann et al. (2017)

(continued)

Table 9.1 (continued)

Virus		Mosquito		Trial (all o.f.)	EIP (days)	Virus detection method	TR (%)/no. of colonies	Result	Reference
	Origin[a]	Species	Origin/no. field/lab/NA	Temperature (°C)/relative humidity (%)				Vector competent (yes/no)	
ZIKV	Asian genotype?	Culex torrentium	Germany/1NA	18/80 27/80	14 14	Vi Vi	0 0	N N	Heitmann et al. (2017)
ZIKV	Asian genotype	Culex pipiens	Italy/1f	26±1/70	14	Vi	0	N	Boccolini et al. (2016)

The transmission rate (TR) is defined as the proportion of mosquitoes with virus-infected saliva or salivary glands (RNA or infectious virus) with respect to the number of mosquitoes with infected body and is considered the most reliable method to investigate the competence of a vector to transmit a virus (except for direct virus transmission to vertebrate hosts by infected mosquitoes). Therefore, and for reasons of clarity, infection and dissemination rates were omitted

b, biotype, EIP extrinsic incubation period (days after infection with virus-containing blood meal) is shown for all studies at around an EIP of 14 days to allow comparison of all studies, FFA foci-forming assay (quantification by FFU/mL), g genotype, IFA immunofluorescence assay: evaluate disseminated infection rate (surviving females were tested for the presence of RVFV on head squashes by IFA after an EIP of 14 days), NA information not available, no. field/lab/NA number of different populations tested collected in the field (f)/obtained from a laboratory colony (l)/unknown (NA), o.f. oral feeding (all mosquitoes in the presented studies were fed with a blood meal containing infectious virus), PCR/Vi detection of virus genome (RNA) by polymerase chain reaction (PCR) or detection of infectious virus by virus isolation (Vi) assay in saliva or salivary glands, TR (%) transmission rate (TR is defined as the number of mosquitoes with virus-positive saliva per number of virus-positive mosquito bodies and (Heitmann et al. 2017) provides the most important information about the vector competence of a mosquito since only virus transmission by saliva during an insect bite is infectious for the vertebrate host is quantified), NL The Netherlands, UK United Kingdom, Ø mean temperature value

[a]By species, genus, family, order (International Committee on Taxonomy of Viruses (ICTV), https://talk.ictvonline.org/taxonomy, 2017): CHIKV, Chikungunya virus, Alphavirus, Togaviridae; DENV, Dengue virus, Flavivirus, Flaviviridae; JEV, Japanese encephalitis virus, Flavivirus, Flaviviridae; RVFV, Rift Valley fever phlebovirus, Phlebovirus, Phenuiviridae, Bunyavirales; USUV, Usutu virus, Flavivirus, Flaviviridae; WNV, West Nile virus, Flavivirus, Flaviviridae; ZIKV, Zika virus, Flavivirus, Flaviviridae

[b]Molecular cDNA clones of JEV genotype 3 strain RP-9 and JEV genotype 5 strain XZ0934 (de Wispelaere et al. 2017) were transfected in HEK293T cells and grown in DF-1 cells

[c]NCBI accession numbers (USUV1, KF055442; USUV2, KF055441; USUV3, KF055440; Italy/2009/FIN, KF234080 (lineage 1 according to Lim et al. 2013)): NY-99, DQ211652; USUV Bologna/09, HM569263; WNV Gr-2010 lineage, HQ537483.1; JEV genotype 3 strain Nakayama, EF571853

[d]Virus RNA detection (Huber et al. 2014a, b) or virus staining by IFA (Moutailler et al. 2008) in whole head samples (not directly in saliva or salivary glands)

[e]Virulent RVFV strain ZH548, Egypt & avirulent Clone 13, Central African Republic, Bangui

contrast, a considerably higher TR was found in *Aedes vexans* originating from a German colony infected with an avirulent RVFV strain (Clone 13) (TR of 25%) compared to a virulent RVFV strain (ZH548) (TR of 8.3%) (Moutailler et al. 2008).

In European mosquito populations, transovarial transmission has only been investigated by Fortuna et al. (2015b) in four different *Culex pipiens* populations collected in Italy and experimentally infected with WNV. However, vertical transmission could not be confirmed in their offspring, although all four populations showed similar TR in their saliva (TR of 37–47%) and were therefore vector competent (Fortuna et al. 2015a) (Table 9.1). In contrast, transovarial transmission was found for WNV in *Culex vishnui* in India (Mishra and Mourya 2001) and for an insect-specific flavivirus (*Culex flavivirus*) by American *Culex pipiens* (Saiyasombat et al. 2011). *Bagaza virus* (*Flaviviridae)* was transovarially transmitted by *Culex tritaeniorhynchus* from India, but not by *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes (Sudeep et al. 2013). Various studies in non-European countries confirmed the possibility of natural transovarial transmission by *Aedes aegypti* for different viruses such as DENV and ZIKV by analysis of immature mosquito stages (Gutiérrez-Bugallo et al. 2017; Li et al. 2017; Velandia-Romero et al. 2017). A high percentage of transovarial transmission of DENV (54.7% of immature stages in households) together with the possibility of transmission by the vector without a prior blood meal has been suggested a possible explanation for the persistence of DENV in (rural) areas (Velandia-Romero et al. 2017). However, the impact of transovarial transmission for DENV in other regions was found negligible, scrutinizing the elimination of larvae as intervention methods (Angel et al. 2016). On the other hand, elimination of larvae is not considered a powerful method for vector control (Pfeffer 2015) (see section vectorial capacity).

9.2.3 *Vectorial Capacity*

The vectorial capacity (VC) is defined as the efficiency of a mosquito species to serve as a vector for a given pathogen and can be estimated using calculations of the basic reproductive rate (R_0). VC is an entomological restatement of R_0 of a pathogen (Kramer 2016; Schaffner and Mathis 2014). R_0 is defined as the number of secondary infections expected to occur from the introduction of a single infection in a naïve population (Kramer 2016), and a key method to understand disease transmission. A major epizootic outbreak and spread of disease within a population are expected if $R_0 > 1$, while minor disease outbreaks that become extinct are expected if $R_0 < 1$. R_0 can be used to plan strategies for control of epizootics but also to estimate, quantify, and compare the outcome of control measures (Pfeffer 2015; Weesendorp et al. 2011). Out of different published equations, the following was proposed by Kramer (2016) and Pfeffer (2015):

$$R_0 = \text{VC} = ma^2 \left(\text{IR}^* \text{TR} \right) p^t / -\ln \left(p \right)$$

VC, vectorial capacity (R_0)

m, vector density in relation to the vertebrate host

a, probability that vector feeds on a host in 1 day (i.e., host preference index * feeding frequency)

p, probability that vector survives one day

t, duration of extrinsic incubation period (EIP) in days (latency period)

IR, infection rate (proportion of vectors infected after feeding on an viremic host)

TR, transmission rate (proportion of infected vectors that are able to transmit the virus to a host)

(IR * TR), vector competence (proportion of vectors ingesting an infective blood meal that are later able to transmit the infection to a host)

$1/-\ln(p)$, duration of the vector's life in days after surviving the EIP (recovery rate)

Accordingly, viral factors are of major importance: a rapid dissemination of a virus from the midgut to the salivary glands would reduce the EIP and, hence, at the same time prolong the duration of the vector's life after surviving the EIP ($=1/-\ln(p)$). In contrast, host feeding (a), vector longevity (p), and EIP (t) would have a more powerful impact on VC (as square or component), while the vector-to-host density relation (m) and vector competence (IR * TR) of a mosquito population would have a linear and therefore weak effect on VC (Kramer 2016).

The control of malaria (caused by parasitic *Plasmodium* spp.) is a vivid example to demonstrate the power or weakness of different control strategies. Control of mosquito larvae affects the vector-host proportion, but a reduction of larvae (m) by 50% only results in a 50% reduction of the VC. However, a reduction of the daily survival time of mosquito vectors of *Plasmodium* (p) by 50% results in a 1000 times lower proportion of mosquitoes that transmit malaria since a reduction of p (survival time) has a direct effect on EIP (t) and the recovery rate ($1/-\ln(p)$) (Pfeffer 2015).

9.2.4 Outcome of Experimental Vector Competence Studies by Virus Species

9.2.4.1 CHIKV

Aedes albopictus, one of the most invasive mosquitoes now endemic across southern Europe, was the main vector for the initial CHIKV outbreak in Italy in 2007 (Bonilauri et al. 2008). *Aedes aegypti*, another primary vector of CHIKV, was introduced in Madeira (Portugal) in 2005 (CDC 2017; Sigfrid et al. 2017). Further autochthonous chikungunya outbreaks were reported in France in 2010 and 2014 (Delisle et al. 2015; Gould et al. 2010). The risk of CHIKV introduction and spread in Europe are highlighted by recent autochthonous outbreak of chikungunya in Italy and spread to France in 2017 (CDC 2017). Bioassays for vector competence studies have been conducted with four different *Aedes albopictus* field populations

collected in Italy ($n = 2$) and Corsica, France ($n = 2$) (Moutailler et al. 2009; Talbalaghi et al. 2010). In both experiments, mosquitoes were infected with a CHIKV strain from the island La Réunion and kept at 28 °C. The TR was approximately between 10 and 80% up to 100% (Moutailler et al. 2009; Talbalaghi et al. 2010). These results are similar to the TR (61%) measured in a US *Aedes aegypti* strain infected with another CHIKV isolate (Blagrove et al. 2016). However, the latter experiment was conducted at a considerably lower temperature (21 °C). In comparison to the main vectors of CHIKV, the mosquito species *Aedes detritus* endemic to the UK, and a possible vector of JEV, RVFV, and WNV (Table 9.1), was CHIKV-infected and kept under the same experimental settings as *Aedes aegypti* (Blagrove et al. 2016). In contrast to *Aedes aegypti*, *Aedes detritus* was not susceptible to CHIKV infection, at least in this experimental setting (Blagrove et al. 2016). However, higher temperatures during the infection experiment or during the maturation of insects may affect their vector competence (Kramer 2016; Lourenço-de-Oliveira et al. 2013; Mellor 2009) for CHIKV. Hence, further vector competence studies are needed for abundant European mosquito species such as *Culex pipiens* and *Aedes vexans* to analyze their vector competence for CHIKV. A study of dissemination rates (DR) in Italian populations of *Anopheles maculipennis* (0%), *Aedes vexans* (7.7%), and *Culex pipiens* (0–33%) after CHIKV infection showed low susceptibilities suggesting a negligible role of these European mosquito species for CHIKV transmission (Talbalaghi et al. 2010).

9.2.4.2   DENV

A large increase in dengue fever cases has been experienced around the globe in the past decades. Between 2010 and 2014, repeated sporadic or large outbreaks have been reported in over 20 European countries (Kampen and Werner 2015; Sigfrid et al. 2017; WHO 2017). *Aedes aegypti* and *Aedes albopictus* are considered the two main vectors of DENV. Infection by one of the four DENV serotypes (DENV-1 to DENV-4) only mediates partial and temporary cross-immunity. Even more, additional infections with other serotypes can lead to severe dengue (Dejnirattisai et al. 2010). Despite to permanent risk of DENV introduction to Europe, only a few studies on the vector competence of European mosquito species have been conducted. One study uses British *Aedes detritus* and tropical *Aedes aegypti* mosquitoes for DENV infection and kept the mosquitoes at a low ambient temperature of 21 °C and 70% RH after infection to simulate low temperate temperatures of Great Britain (Blagrove et al. 2016). Similar to the results of CHIKV infection in these mosquito strains, *Aedes detritus* was not susceptible to DENV-2, while *Aedes aegypti* showed a high TR of 70%. Talbalaghi et al. (2010) and Moutailler et al. (2009) investigated dissemination rates (DR) of Italian and Corsican (France) *Aedes albopictus* populations after infection with DENV-2, but not TR. Italian *Aedes albopictus* (14–39%) and Corsican *Aedes albopictus* (13–69%) kept at 28 °C for 14 days showed similar DR. Because of intrinsic barriers in the mosquito potentially interfering with transmission, the TR as a proxy for vector competence is not necessarily similar to

DR. Thus, vector competence for European mosquito populations of *Aedes albopictus* is not confirmed yet, but is likely considering the global role of *Aedes albopictus* as vector of DENV. Further studies are needed to investigate the vector competence for various potential European mosquito vectors and the four DENV serotypes.

9.2.4.3 JEV

JEV is an exotic flavivirus to Europe. However, recent detection of fragmented JEV-RNA in Italian *Culex pipiens* mosquitoes and birds caught in 2010 indicated a sporadic introduction of JEV to Europe, although complementary studies to confirm the presence of JEV in Europe are required (Platonov et al. 2012; Ravanini et al. 2012; Zeller 2012). Several groups therefore aimed to investigate the vector competence of mosquito species endemic (*Aedes detritus* and *Culex pipiens*) or invasive (*Aedes albopictus*, *Aedes japonicus japonicus*) to Europe. While *Aedes albopictus* by now commonly occurs in large parts of Europe (in particular in Southern Europe), *Aedes japonicus japonicus* occurs considerably less frequent in Europe. However, this mosquito species is adapted to temperate regions, has been established in a few regions of Germany since 2008 (Kampen and Werner 2015), and was shown competent for JEV replication (Huber et al. 2014a). All four European mosquito species—*Aedes detritus* collected in the UK, *Culex pipiens* and *Aedes japonicus japonicus* collected in Germany, as well as *Aedes albopictus* collected in France that were orally infected with JEV strains of genotype 3 or 5 (Table 9.1)—were found competent for JEV transmission (de Wispelaere et al. 2017; Huber et al. 2014a; Mackenzie-Impoinvil et al. 2015). De Wispelaere et al. (2017) used two cDNA clones of field strains after their rescue in cell culture, while all other groups used field strains. *Aedes albopictus*, *Aedes japonicus japonicus*, and *Culex pipiens* species were kept at 25 or 26 °C and 80–85% RH, simulating intermediate to diurnal summer temperatures of Mediterranean Europe. TR ranged between 12 and 63% for *Aedes albopictus* and *Culex pipiens*. For *Aedes japonicus japonicus*, only the DR in the whole head (analyzed by PCR) was investigated, which was considerably higher (100%) (Huber et al. 2014a) compared to the TR of JEV found for the other mosquito species. Therefore, the high DR cannot necessarily be used to draw conclusions for the TR, which requires analyses of saliva or at least salivary glands (see section barriers). The study was included in this review since no other studies of vector competence for JEV in European *Aedes japonicus japonicus* mosquito populations have been conducted so far. The vector competence of local (temperate) British *Aedes detritus* mosquitoes was comparatively analyzed using 23 or 28 °C and a RH range of 70–90%. Interestingly, *Aedes detritus* mosquitoes were found competent at both temperatures, although the RT was markedly lower at 23 °C (TR of 3%) compared to 28 °C (TR of 17%). Interestingly, similar TRs were obtained for *Culex quinquefasciatus*, a tropical mosquito previously incriminated as vector for JEV (Mackenzie-Impoinvil et al. 2015). In summary, the results of the vector competence studies of JEV in three commonly occurring mosquito species in

Europe suggest that JEV transmission is possible in various European countries especially during warm summer nights and in Mediterranean Europe. Complementary studies are necessary to determine the vector competence of different *Aedes japonicus japonicus* populations invasive in Europe for JEV. The results of the vector competence studies together with the recent detection of fragmented RNA of a JEV or a related virus highlight the need for comprehensive surveys of JEV in different mosquito species in Europe.

9.2.4.4 RVFV

RVFV is an arbovirus mainly transmitted by a large number of different mosquito species to different mammals including humans in Africa. Multiple outbreaks of RVFV outside Africa, particularly in countries bordering the Mediterranean Sea, point to a high probability of RVFV outbreaks in Europe. Key drivers of seasonally high numbers of RVF disease outbreaks are heavy rainfalls following periods of drought that suddenly increase vector density (due to rain associated hatching of larvae to imago). The high vector density at water holes leads to a high probability of infection of susceptible vertebrate hosts that regularly visit water holes for drinking. The possibility of transovarial transmission of RVFV to the mosquito offspring as reported by Linthicum et al. (1985) contributes to efficient transmission of this virus (Brustolin et al. 2017; Moutailler et al. 2008).

Vector competence studies for RVFV in European mosquito species are scarce. Oral infection of Spanish *Aedes albopictus*, *Culex pipiens* biotype *molestus*, and hybrid *Culex pipiens* biotype *pipiens* x *molestus* with an South African RVFV strain resulted in the release of infectious virus transmission in saliva of a few individuals belonging to the species *Aedes albopictus* and the hybrid *Culex pipiens* biotype *pipiens* x *molestus* (exact proportion of the TR was not given) but not of the species *Culex pipiens* biotype *molestus* (Brustolin et al. 2017). The midgut barriers of infection (MIB) and escape (MEB) were comparatively analyzed in the species *Culex pipiens* biotype *molestus* and the hybrid species by virus isolation. Two different viral doses were used for oral infection (5.7 $\log_{10}TCID_{50}$/mL or 5.7 $\log_{10}TCID_{50}$/mL). Interestingly, while the lower and higher doses resulted in infection of the MIB in both species (IR of 7–20%), the MEB was only overcome in hybrid *Culex pipiens* biotype *pipiens* x *molestus* after infection with the higher virus dose (DR of 66.6%), but not in the species *Culex pipiens* biotype *molestus* (0%). A similar dependence of the viral dose on the infection and escape of midgut cells was previously reported for BTV in *Culicoides* (Mellor 2009). On the other hand, *Culex pipiens* biotype *molestus* is generally refractory to infection with various other viruses (WNV lineages 1 and 2, and ZIKV) (Brustolin et al. 2017; Heitmann et al. 2017) (Tables 9.1 and 9.2). Moutailler et al. (2008) studied various European mosquito species regarding their vector potential for RVFV by analyzing virus in head squashes by immunofluorescence assay, and hence the DR but not TR. At 14 days postinfection, *Aedes vexans* showed a considerably lower DR in virulent RVFV (ZH548, 8.3%) compared to an avirulent strain (Clone13, 25%) (Moutailler

Table 9.2 Vector competence of different mosquito-vector species for endemic and emerging pathogens in Europe

Mosquito species	Experimentally confirmed vector competence[a]	Experimentally confirmed lack of vector competence[a]	Collective field and experimental results[b]
Aedes albopictus	SINV[c], CHIKV, JEV, RVFV, WNV L1, WNV L2, ZIKV	USUV	CHIKV, DENV
Aedes caspius	RVFV	–	WNV, SINV, TAHV, USUV
Aedes detritus	JEV, RVFV, WNV L1	CHIKV, DENV	USUV
Aedes japonicus japonicus	JEV, WNV L1	WNV L1	WNV, SINV, TAHV, USUV, RVFV
Aedes vexans	RVFV	–	WNV, SINV, TAHV, USUV, RVFV
Culex pipiens	JEV, RVFV, USUV, WNV L1	WNV L2, ZIKV	WNV, SINV, TAHV, USUV, RVFV
Culex p.p. b. *molestus*	–	RVFV, WNV L1, WNV L2, ZIKV	–
Culex p.p. b. *pipiens*	–	ZIKV	–
Culex p.p. b. *pipiens* x b. *molestus* (hybrid)	RVFV, WNV L2	WNV L1, ZIKV	–
Culex torrentium	–	ZIKV	SINV

[a]Summary of vector competence studies by mosquito species (as described in Table 9.1), and, for comparison, [b]collective results of European field studies and experimental studies as reviewed by Kampen and Werner (2015), Húbalek (2008), and Nikolay (2015)
L lineage, *p. pipiens*, b. biotype, - no information available
SINV, *Sindbis virus* (*Alphavirus, Togaviridae*); TAHV, *Tahyna virus*, infraspecies of *California encephalitis virus, Peribunyaviridae*); [c]result of experimental infection of *Aedes albopictus* with SINV by Dohm et al (1995); references for [a] according to Table 9.1: CHIKV, *Chikungunya virus* (Talbalaghi et al. 2010; Moutailler et al. 2009; Blagrove et al. 2016); DENV, *Dengue virus* (Blagrove et al. 2016); JEV, *Japanese encephalitis virus* (Huber et al. 2014a, b; Mackenzie-Impoinvil et al. 2015; de Wispelaere et al. 2017); RVFV, *Rift Valley fever phlebovirus* (Brustolin et al. 2017; Moutailler et al. 2008); USUV, *Usutu virus* (Puggioli et al. 2017; Fros et al. 2015b); WNV, *West Nile virus* (WNV L1: Brustolin et al. 2016; Fortuna et al. 2015a; Fortuna et al. 2015b; Huber et al. 2014a, b; Wagner et al. 2018; Blagrove et al. 2016; Fros et al. 2015a; WNV L2: Brustolin et al. 2016; Fros et al. 2015a; Fros et al. 2015b); ZIKV, *Zika virus* (Heitmann et al. 2017; Jupille et al. 2016; Di Luca et al. 2016; Boccolini et al. 2016); Werner et al. 2015; Húbalek 2008)

et al. 2008). In contrast, for the three European mosquito species, namely, *Aedes detritus*, *Culex pipiens* (France), and *Culex pipiens* (Cyprus) infected with both RVFV strains, DR were markedly higher after infection with the virulent ZH548 (13–30%) compared to the avirulent Clone 13 strain (0–14%) (Table 9.1). In the

French colonies of *Aedes caspius* (7%) and *Culex pipiens* (9%) infected with the avirulent Clone 13 RVFV strain, DR were similarly low (7 and 9%, respectively) (Moutailler et al. 2008). In addition to vector competence of the European mosquitoes, results were compared with field strains of different *Aedes* and *Culex* species from different African and Asian countries. In general, similar dissemination of the virus is found in all tested species compared with the DR results of the European mosquito species, except for *Aedes aegypti*. *Aedes aegypti* showed a considerably higher DR of 20–90% for the virulent RVFV ZH458 and 24–73% for the avirulent Clone 13 strain suggesting that transmission of RVFV by *Aedes aegypti* is more efficient (Moutailler et al. 2008). On the other hand, mosquitoes belonging to the *Culex pipiens* complex are considered efficient vectors of RVFV in Africa, and virus isolation of RVFV from at least 40 mosquito species (Moutailler et al. 2008) indicates that the broad variety of competent vectors of RVFV primarily contributes to the efficient transmission of this virus in highly diverse habitats and climatic regions. The demonstration of vector competence of Spanish field populations of *Culex pipiens* and *Aedes albopictus* for RVFV and the potential vector competence of other European mosquitoes indicate that autochthonous outbreaks of RVFV are possible in Southern Europe.

9.2.4.5 Usutu Virus

In a comprehensive field study of USUV infection in different mosquito species in Italy from 2009 to 2012, a substantial incidence of *Aedes albopictus* mosquitoes PCR-positive for USUV was found. However, USUV was not detected in any of the *Aedes albopictus* specimens collected in 2013 (Puggioli et al. 2017). Experimental infection of *Aedes albopictus* collected in the field in Italy with any of the three Italian virus strains (of 2011) and incubation at 28 °C and 80% RH showed RNA in a single individual after an EIP of 7 days, but no mosquitoes were found PCR-positive after an EIP of 14 days. Therefore, Puggioli et al. (2017) suggested that *Aedes albopictus* plays a negligible role in the epidemiology of USUV, but further studies are necessary using different experimental parameters. In contrast, *Culex pipiens* orally infected with USUV strain Bologna/09 showed a high vector competence (TR of 69%) at an EIP of 14 days at 28 °C and 60% RH, which is significantly higher compared to TR found for *Culex pipiens* infected with WNV lineage 2 strain Gr-2010 (TR of 33%) by the same group (Fros et al. 2015b). A considerable dependence on temperature was found comparing infection rates of *Culex pipiens* mosquitoes kept at 60% RH and the three different temperatures 18 °C (TR of 11%), 23 °C (TR of 53%), and 28 °C (TR of 90%). Since these three different temperatures represent the mean diurnal summer (July–August) temperature in North-Western Europe, an intermediate temperature, and the mean diurnal summer temperature for Mediterranean Europe, respectively, it can be assumed that particularly in Southern Europe, the transmission rate of USUV by *Culex pipiens* is considerably higher (Fros et al. 2015b). In a comprehensive field study of USUV occurrence in different

mosquito species in Germany, USUV was detected or isolated from *Culex pipiens* (Jost et al. 2011a; Sieg et al. 2017). In field studies in Italy (Calzolari et al. 2012; Mancini et al. 2017) and other countries (reviewed in Nikolay (2015)), additional mosquito species were found PCR-positive for USUV, including *Culex pipiens s.l.*, *Aedes albopictus*, *Aedes caspius*, *Aedes detritus*, *Anopheles maculipennis*, and *Culiseta (Cs.) annulata*. Similar to the results of the German studies (Jost et al. 2011a; Sieg et al. 2017), the cumulative results of the Italian field studies confirm that *Culex pipiens* likely is most involved in USUV circulation in Italy (Calzolari et al. 2012; Mancini et al. 2017) and in other European countries.

9.2.4.6 West Nile Virus

In Europe, *Culex pipiens* is considered the main vector of WNV, but other species such as *Aedes albopictus* (Fortuna et al. 2015a), *Aedes detritus*, or *Aedes japonicus japonicus* (Wagner et al. 2018) may also act as competent vectors. Therefore, several research groups investigated the vector competence of these mosquito species in comparison to the main European vector *Culex pipiens* for WNV lineage 1 and 2 strains by using field and laboratory mosquito colonies collected in different European countries. Huber et al. (2014a) did not find replication of North American WNV lineage 1 strain NY-99 in a German *Aedes japonicus japonicus* population after artificial infection, while Wagner et al. (2018) found the *Aedes japonicus japonicus* populations collected in the neighboring country Switzerland susceptible for the same WNV strain and the Italian strain Italy/2009/FIN. *Aedes detritus*, a mosquito species endemic in the UK, were kept at 21 °C and 70% RH (according to climatic conditions in the UK during warmer seasons) during the experiment and were found competent for WNV strain NY-99 infection under these conditions (Blagrove et al. 2016). As expected, *Culex pipiens* endemic in Switzerland were found competent for the replication of WNV strain NY-99 (Wagner et al. 2018). A comparison of vector competence for European WNV lineages 1 and 2 strains was conducted by Brustolin et al. (2016) and Fros et al. (2015b). In contrast to other studies, Brustolin et al. (2016) used a fluctuating temperature regimen (mean of 21.3 °C at night and mean of 27.7 °C during the day, at 70% RH) to mimic natural conditions. For the comparative study of WNV line 1 and 2 strains, *Aedes albopictus*, *Culex pipiens pipiens* biotype *molestus*, and *Culex pipiens pipiens* hybrids of biotypes *pipiens* and *molestus* were collected in the field in Spain and orally infected with European WNV lineage 1 (France 2001) or 2 (Italy 178907/2013). The *Culex pipiens* hybrid was competent for lineage 2 but refractory to WNV lineage 1 (Brustolin et al. 2016). In contrast, *Aedes albopictus* was found competent for both strains (Brustolin et al. 2016). Similarly, a field colony of *Aedes albopictus* collected in Italy and orally infected with the European Sardinia 2011 lineage 1 strain Ma V3 kept at 27 °C and 70% RH showed a high vector competence (TR of 50%) (Fortuna et al. 2015b). A possible reason for a broader vector competence, more efficient transmission of arboviruses, and outbreak establishment might be

that *Aedes albopictus* has a higher genetic variability due to independent and transcontinental introductions (Manni et al. 2017), which could therefore facilitate the adaption of this mosquito species to different regions and climates. Considerable genomic variations in *Aedes japonicus japonicus* due to similar reasons were also suggested by Kampen and Walther (Kampen and Werner 2014; Zielke et al. 2014, 2015, 2016). Fros et al. conducted vector competence studies with a laboratory colony of *Culex pipiens* collected in the Netherlands. After infection with the WNV lineage 1 strain NY-99 and the European lineage 2 strain Gr-2010 and maintenance at 23 °C (mean average temperature in Central Europe) and 28 °C (Mediterranean mean diurnal summer), a similar vector competence for both lineages and a slightly higher transmission rate at a higher temperature (TR of 33% compared to 24%) were found (Fros et al. 2015a, b). Interestingly, the vector competence and dissemination rate of these North-West European *Culex pipiens* was similarly high for both the NY-99 and Gr-2010 strains at 23 °C, while mosquitoes of North American origin infected with the same strains showed a significantly lower transmission rate for the WNV lineage 2 strain (Fros et al. 2015a). Unfortunately, the biotype of the *Culex pipiens* was not described to evaluate whether these mosquitoes were hybrids that may inherit a higher vector competence compared to *Culex pipiens* biotype *molestus* as described by Brustolin (Brustolin et al. 2016).

9.2.4.7   Zika Virus

ZIKV has been circulating in Africa and South-East Asia for over 65 years. However, during the recent ZIKV endemic in the Americas, this Asian ZIKV genotype has been linked to different phenotypic characteristics (including congenital malformation and neurological disorders in humans, higher infection rates in *Aedes aegypti*) compared to the African ZIKV genotype (Willard et al. 2017). A risk analysis of Gardner et al. (2017) revealed that the vector status of *Aedes* species determines geographical risk of autochthonous ZIKV establishment. While the risk is geographically limited if *Aedes aegypti* is the only competent ZIKV vector, vector competence of *Aedes albopictus* would pose a risk of local establishment in all American regions including Canada and Chile, much of Western Europe, Australia, New Zealand, and South and East Asia, with a substantially increase in the risk of ZIKV outbreaks in Asia (Gardner et al. 2017). To estimate the risk of different mosquito species in different climatic regions, European *Aedes albopictus* were collected from the field in Italy, France, and Germany and experimentally infected with ZIKV belonging to the Asian genotype. *Aedes albopictus* were found competent at temperatures between 26 and 28 °C, but refractory to ZIKV at 18 °C (Di Luca et al. 2016; Heitmann et al. 2017; Jupille et al. 2016). In contrast, *Culex pipiens* collected in Italy and kept at 26 °C and 70% RH (Boccolini et al. 2016) as well as *Culex pipiens* biotype *molestus* and biotype *pipiens* and *Culex torrentium* collected in Germany incubated at 18 or 27 °C and 80% RH (Heitmann et al. 2017) were not found competent vectors of the Asian ZIKV genotype.

9.2.5 Lessons Learned by Experimental Vector Competence Studies

In summary, the varying results of the research groups regarding the proportion of mosquitoes of the same species that were found competent for WNV transmission (Table 9.1) may be due to considerable variations in specific mosquito genotype and virus genotype interactions (Lambrechts 2010; Lambrechts et al. 2009a). A considerable genetic variability in *Aedes albopictus* and *Aedes japonicus japonicus* due to independent and transcontinental introductions (Kampen and Werner 2015; Manni et al. 2017) can result in a broader vector competence, more efficient transmission of arboviruses, and regional outbreaks. A meta-analysis of laboratory experiments with DENV indicated that colonization of *Aedes albopictus* over a few generations might result in an increase of their susceptibility to DENV infection (Lambrechts 2010).

On the other hand, the effect of virus genotypes, serotypes, or lineages may be underestimated or overestimated regarding virulence and transmissibility for different mosquito populations of the same species. Vertebrate host factors such as differences in resistance to infection or low viremia may considerably impact virus transmission between hosts (Húbalek 2008; Reisen and Hahn 2007). Adaptation of new viruses to local hosts and vectors by initial positive (diversifying) selection with more virulent quasispecies, followed by negative (stabilizing) selection driven by strong evolutionary constraints, is reported for BTV (Boyle et al. 2012, 2014; Maclachlan et al. 2009; Schulz et al. 2016). For example, *Culex pipiens* populations occurring in North America showed a significantly lower transmission rate for a WNV lineage 2 strain compared to North-Western European *Culex pipiens* species, while a similar transmission rate was found for WNV lineage 1 (Fros et al. 2015a). However, even specific combinations of isofemale families and viral isolates may affect quantity of dissemination within mosquito vectors (Lambrechts et al. 2009a), challenging the validity and relevance of laboratory experiments with single virus-mosquito combinations (Lambrechts et al. 2009a). Furthermore, differences in mortality rates of virus-infected mosquitoes might be due to virus factors (see section virus adaptation to mosquitos). *Aedes albopictus* infected with CHIKV died a few days earlier than non-infected mosquitoes, while the primary vector *Aedes aegypti* survived the infection due to antiviral immune response (see section immune response against arboviruses). A higher frequency of cytopathological changes in salivary glands has been reported in WNV-infected mosquitoes (Girard et al. 2007). Furthermore, fast virus dissemination from the midgut impacting the duration of EIP, low mortality rate, and differences in feeding behavior influence the vectorial capacity of a vector (see section vectorial capacity). Interestingly, *Aedes aegypti* infected with DENV showed a significantly prolonged probing time and enhanced feeding frequency (Platt et al. 1997).

Therefore, the vector competence of various vector genotype and virus genotype combinations by studying different populations over time and space (from different regions/countries of interest) may result in an average and collective experience to

allow an estimation of vectorial capacity of a mosquito species from different areas and over time (Fonseca 2016). In addition, a bias in results of vectorial capacity due to variations in methodologies used by different research groups may be mitigated by analyses of similar virus genotype and mosquito genotype combinations (Lambrechts 2010; Lambrechts et al. 2009a). On the other hand, harmonization of methods (e.g., temperature regimes) and analyses of experiments (representation of proportions of transmission rates by species) as well as the meticulous description of the origin and taxonomy of the used mosquito vectors and virus strains would be most valuable in terms of comparability and reproducibility. In a considerable number of studies, *Culex pipiens* was only superficially taxonomically classified. However, comparison of results of experimental infection of the *Culex pipiens* biotype *pipiens*, *Culex pipiens* biotype *molestus*, and hybrids of both forms revealed considerable differences in their susceptibility to different virus species and lineages (RVFV, WNV lineages 1 and 2, and ZIKV) under equal or similar experimental conditions (Brustolin et al. 2016, 2017; Heitmann et al. 2017) (Table 9.1) insofar that the parental forms *molestus* and *pipiens* of *Culex pipiens* seem to be refractory to the so far tested viruses (Table 9.2), while hybrids of *Culex pipiens* biotype *pipiens* and *molestus* were found competent for RVFV and WNV lineage 2 (Tables 9.1 and 9.2).

Change in climate, land use, genetic diversity within mosquito species in combination with a rapid arboviral adaptation to alternative mosquito, and vertebrate hosts constitute a dynamic system that can substantially and rapidly change the epidemiological patterns of a viral disease as well as the disease expression in vertebrate hosts and therefore the impact on animal welfare and economy of affected countries (Kramer 2016; Lambrechts 2010; Schulz et al. 2016).

9.3 How Do Viruses and Vectors Interact to Facilitate Transmission?

Arboviruses can efficiently replicate in evolutionary distinct hosts, such as mosquitoes and humans; yet they seem to depend on specific mosquito vectors for transmission. The intrinsic factors that determine whether a specific mosquito can transmit a given virus (vector competence) remain poorly understood. Major factors defining vector competence of mosquito species are (1) the control of viral replication by the mosquito to an extent that the mosquito itself is not affected by the virus, (2) virus adaptation to the mosquito to increase viral replication, and (3) the microbiome in the insect vector (illustrated in Fig. 9.3). Within this part, a brief overview of these factors will be given, but since these factors are subject to intense research these days, not all details can be given in the frame of this chapter. For more detailed information please refer to recent reviews (Blair and Olson 2015; Donald et al. 2012; Johnson 2015; Sim et al. 2014).
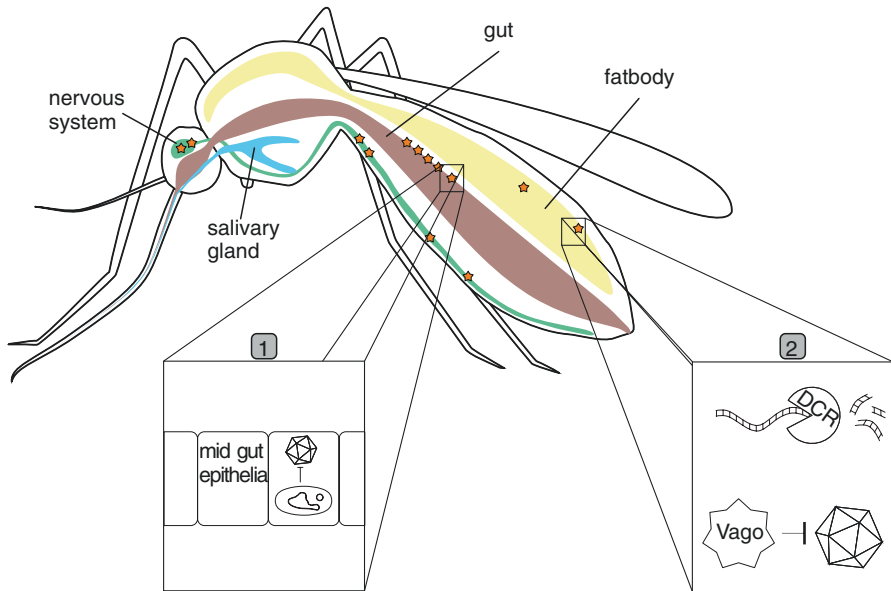
Fig. 9.3 Intrinsic factors that interfere with the vector competence of mosquitoes. The vector competence of a certain mosquito species is characterized by several factors: firstly, the ability of the virus to overcome the midgut barrier (midgut infection and escape barrier), secondly the ability of the virus to replicate in various tissues of the insect host, and most importantly the efficient dissemination of infectious viral particles to the saliva (salivary gland infection and escape barrier). The virus replication in the mosquito midgut is regulated by the gut microbiota represented by the *Wolbachia* endosymbiont (1) which can interfere with the virus replication by various ways including immune priming and completion for resources. After the dissemination of the virus to different mosquito organs such as the fat body and endothelial cells, the virus starts to replicate in these different tissues. The virus replication can trigger several antiviral pathways such as the RNAi pathways represented by Dicer cleavage and the inducible immune responses represented by the induction of *Vago* (2)

9.3.1 Immune Response in Insects Against Arboviruses

9.3.1.1 RNAi Responses

Lacking an adaptive immune system, insects depend on different immune mechanisms for antiviral defense. Using the model insect *Drosophila melanogaster*, it has been demonstrated that RNA interference (RNAi) pathways are crucial to control various *Drosophila* viruses and also metazoonotic viruses such as SINV, WNV, *v Vesicular stomatitis virus* (VSV), and DENV (Chotkowski et al. 2008; Galiana-Arnoux et al. 2006; Mukherjee and Hanley 2010; van Rij et al. 2006; Wang et al. 2006; Zambon et al. 2006). The exogenous (antiviral) siRNA pathway (exoRNAi) is initiated by recognition and cleavage of long double-stranded (ds) RNA, deriving from viral replication intermediates or secondary RNA structures in viral genomes, by the RNaseIII enzyme Dicer-2 (Dcr-2). The resulting 21 nucleotide (nt)-long

virus-derived small interfering RNAs (viRNAs) are then subjected to a multiprotein RNA-induced silencing complex (RISC). In this complex, the major component Argonaute-2 (Ago2) together with one strand of the viRNA initiates the sequence-specific degradation of viral genomes or transcription products (Liu et al. 2006; van Rij et al. 2006). Survival experiments in *Drosophila* lacking the key components of a functional exoRNAi response have demonstrated the exoRNAi-mediated control of arbovirus replication is crucial for the insects' survival (Dietrich et al. 2017a; Kemp et al. 2013; Mueller et al. 2010; Mukherjee and Hanley 2010). The sequencing of full genomes of *Aedes aegypti* (Nene et al. 2007), *Culex quinquefasciatus* (Arensburger et al. 2010), and *Anopheles gambiae* (Holt et al. 2002) enabled the identification of orthologues of *Dcr-2* and *Ago2* in three important vector mosquito species (Campbell et al. 2008a) and subsequent description of further *Dcr-2* and *Ago2* orthologues in more vector species such as *Aedes albopictus* (Brackney et al. 2010). Furthermore, the production of viRNAs, a hallmark of exoRNAi pathway induction, has been shown in *Aedes* and *Culex* mosquitoes response to infection of mosquitoes with different arboviruses (Blair and Olson 2015; Brackney et al. 2010; Campbell et al. 2008b; Carissimo et al. 2015; Dietrich et al. 2017a, b; Leger et al. 2013). The full genome sequences further enabled to study the role of antiviral exoRNAi pathways for vector function of these mosquito species. For example, Keene et al. (2004) were able to show that knockdowns of Dicer and Argonaute genes in *Anopheles gambiae* lead to increased replication of ONNV. However, Carissimo et al. (2015) showed that the induction of the exoRNAi pathway is not essential to control the ONNV infection in the midgut and thus speculate that the role of exoRNAi may be more important during dissemination of the infection than at the initial site of infection. In contrast, Khoo et al. showed that the infection of *Aedes aegypti* with *Togaviridae* is controlled by exoRNAi pathways at the level of the midgut barrier (Khoo et al. 2010). The tissue-specific knockdown of Dcr-2 in the midgut leads to enhanced replication and increased viral escape from the midgut (Khoo et al. 2010). The importance of exoRNAi in the defense of *Aedes aegypti* against SINV was further demonstrated by Campbell et al. (2008b), Myles et al. (2008), and Cirimotich et al. (2009) of which the latter study demonstrated that suppression of the exoRNAi pathway leads to reduced survival of infected mosquitoes. The contradicting observations in two different vector species, *Aedes* and *Anopheles*, indicate that, although exoRNAi is accepted as the major antiviral response in insects (Blair and Olson 2015; Kemp et al. 2013), the importance of this response can be tissue- and vector species-specific. The major role of RNAi in *Aedes aegypti* mosquitoes was further underlined by the observations made by Sanchez-Vargas et al. (2009) showing that DENV is controlled by the exoRNAi pathway and that loss of this pathway leads to increased virus replication and a shortened EIP. Besides *Aedes*, *Culex* mosquitoes are major vectors for arboviruses. Despite their importance, less data on exoRNAi pathway induction and function are available for *Culex* mosquitoes. Brackney et al. (2009) demonstrated that WNV infection induces small RNA production in *Culex quinquefasciatus* mosquitoes indicating that the exoRNAi pathway plays a role in these mosquitoes. Also the production of viRNAs in RVFV-infected *Culex quinquefasciatus* mosquitoes (Dietrich et al. 2017a) and the

demonstration of WNV- and USUV-derived small RNAs in *Culex pipiens* mosquitoes (Fros et al. 2015b) are suggestive for an antiviral role of the exoRNAi pathway in *Culex* spp. However, functional evidence as it is presented for *Aedes* and *Anopheles* mosquitoes is currently lacking for *Culex* mosquitoes.

Besides the exoRNAi pathway, the Piwi-interacting RNA (piRNA) pathway can be activated in mosquitoes after infection with arboviruses. This pathway was initially described in *Drosophila melanogaster*, where the expression of transposons in germline cells and ovarian follicle cells is controlled by piRNAs (Brennecke et al. 2007). The 24- to 29-nt-long piRNAs are generated in a Dicer-independent manner and show a characteristic molecular signature (Brennecke et al. 2007; Morazzani et al. 2012; Vodovar et al. 2012). The piRNA pathway is initiated by the long single-stranded precursor RNAs that transcribed from piRNA clusters in the genome (Brennecke et al. 2007). This signal is amplified by the so-called ping-pong amplification loop (Siomi et al. 2011) including the Argonaute-3 (Ago3), Aubergine (Aub), and Piwi proteins (Brennecke et al. 2007; Gunawardane et al. 2007; Saito et al. 2006). In contrast to *Drosophila melanogaster*, the piRNA pathway has undergone an expansion in aedine and culicine mosquitoes with seven Piwi proteins (Piwi1–7) in *Aedes aegypti* and six Piwi proteins in *Culex quinquefasciatus* (Campbell et al. 2008a; Schnettler et al. 2013). This expansion correlates well with the extended role of the piRNA pathway in mosquitoes. Up to date, virus-specific piRNAs have been found in *Aedes* mosquitoes infected with members of all major arbovirus families and orders *Flaviviridae* (DENV), *Togaviridae* (SINV, CHIKV), and *Bunyavirales* (Dietrich et al. 2017b; Hess et al. 2011; Morazzani et al. 2012; Vodovar et al. 2012). The mechanism by which virus-derived piRNAs are induced is still not completely understood, but a recent study has given some insight into the mechanism of virus-derived synthesis in mosquito cells showing its dependence on Piwi5 and Ago3 proteins (Miesen et al. 2015, 2016). In addition, the Piwi4 protein is shown to be essential to control *Semliki Forest virus* (SFV, *Togaviridae*), BUNV, and RVFV infection in *Aedes aegypti* mosquito cells (Dietrich et al. 2017a, b; Schnettler et al. 2013), and Ago3 is essential to control ONNV in *Anopheles gambiae* (Keene et al. 2004).

The role of the third RNAi pathway, the microRNA (miRNA) pathway in arbovirus infection, is less clear, but recent data point to an involvement of miRNAs in virus-vector interactions (extensively reviewed in Asgari (2014)). The microRNA pathway exists in most metazoans and was initially described as a posttranscriptional regulatory mechanism. The miRNAs are produced by a Dicer enzyme (in insects Dicer-1) and incorporated into RISC-containing Argonaute proteins. This miRNA aids the RISC to a target RNA sequence which is complementary to the 5′8 nucleotides (seed region) of the miRNA. In mammals the role of cellular as well as virus-derived miRNA in modulation of virus replication has been long known (Muller and Imler 2007); however, a lack of knowledge persists on the role of miRNA in arbovirus-vector interactions. After publication of whole genome sequences from *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles gambiae*, also miRNAs have been identified (*Aedes aegypti* (Li et al. 2009), *Culex quinquefasciatus* (Skalsky et al. 2010), *Anopheles gambiae* (Winter et al. 2007)). A number of studies reported the differential expression of miRNA in these vector mosquitos

after infection with arboviruses. For example, *Culex quinquefasciatus* miR-989 was downregulated, and miR-92 was upregulated during WNV infection, but the meaning of this regulation remains unclear since no target was yet identified for those miRNAs (Skalsky et al. 2010). In *Aedes aegypti*, the infection with DENV serotype 2 alters the abundance of 35 miRNAs of which some have target sequences in genes linked to signal transduction and the cytoskeleton, but to date, no experimental evidence links these potential miRNA-target interactions to virus-vector interactions (Campbell et al. 2014). In contrast, the downregulation of *Aedes albopictus* miR-252 leads to a 1.5-fold increase of DENV serotype 2 virus replication (Yan et al. 2014). Furthermore, *Aedes albopictus* miR-2940, which was found to be unregulated during WNV infection, positively affects WNV replication through the upregulation of metalloprotease m41 *ftsh* (MetP) (Slonchak et al. 2014). However, knockdown of Ago1, the key protein of the miRNA pathway in *Anopheles* and *Aedes*, does not alter replication of several viruses, whereas knockdown of Ago2 (exosiRNA) or Ago3 (piRNA) pathways has a major impact on virus replication. Thus, the role of cellular miRNAs is not entirely clear and needs further investigation.

9.3.1.2 Inducible Antiviral Responses

A couple of inducible mechanisms have been described in *Drosophila* and mosquitoes during the past years. The Toll and immune deficiency (IMD) pathways, initially characterized for their role in the control of bacterial and fungal infections in *Drosophila* (reviewed in Mussabekova et al. (2017)), are now widely recognized immune pathways in mosquitoes (reviewed by Sim et al. (2014)). In mosquitoes, Toll and IMD pathways are induced after pathogen recognition through peptidoglycan recognition proteins (PGRPs). Subsequent intracellular signaling is induced by Spätzle-MyD88 interaction (Toll) or IMD protein (IMD) which leads to the activation of nuclear factor "kappa-light-chain-enhancer" (NF-kB)-like transcription factors, namely, Rel1A (Toll) and Rel2 (IMD). Both pathways trigger the expression of antimicrobial effectors such as cecropins or defensins. The antiviral role of the Toll and IMD pathway was first shown in *Drosophila* after infection with several viruses (Toll, *Drosophila X virus* (Zambon et al. 2005); IMD, SINV and *Cricket paralysis virus* (Avadhanula et al. 2009; Costa et al. 2009)). In mosquitoes first evidence of a potential involvement of the Toll pathway in antiviral defense came from DENV-infected *Aedes aegypti* mosquitoes where 240 genes including key components of the Toll pathway, e.g., Spätzle, Toll, and Rel1A, were differentially regulated (Xi et al. 2008). A functional role of the Toll pathway was further confirmed in DENV-infected *Aedes aegypti* mosquitoes showing that transient Rel1 activation significantly reduces DENV titers, whereas silencing of MyD88 increased virus replication (Xi et al. 2008). Along this line, the induction of the Toll pathway in *Wolbachia*-infected *Aedes aegypti* is believed to be one way how the bacterium interferes with virus replication (Pan et al. (2012); see also section *Wolbachia* below). The impact of Toll pathway activation in other arbovirus infections and other mosquito species is less well studied. SINV and WNV induce the Toll pathway in *Aedes aegypti*

(Colpitts et al. 2011; Sanders et al. 2005), while the latter fails to induce the Toll pathway in *Culex quinquefasciatus* (Bartholomay et al. 2010). Thus, the induction of the Toll pathway due to virus infection might be mosquito species-specific, or orthologues of the Toll pathway have not been completely characterized in other mosquito species, which could explain the lack of detection (e.g., the WNV-induced transcript *CQ G12A2* in *Culex quinquefasciatus* shares 33% homology with the Toll-like receptor of *Aedes aegypti*; Smartt et al. (2009)). The IMD pathway plays a major role in mosquito antibacterial and antiparasite defense (Dong et al. 2009; Garver et al. 2012; Meister et al. 2005). The antiviral role has only been studied recently and in less detail than the Toll pathway. The upregulation of IMD pathway components was shown for *Aedes aegypti* mosquitoes infected with DENV and SINV (Barletta et al. 2017; Luplertlop et al. 2011; Sanders et al. 2005). First indirect evidence for a functional role for the IMD pathway in virus infection was presented by Sim et al. (2013) who showed that silencing of the pathway leads to enhanced viral replication in DENV-refractory strains of *Aedes aegypti*. However, transient activation of the pathway does not influence DENV infection (Xi et al. 2008). Recent findings by Barletta et al. (2017) point to an indirect role of the IMD pathway by controlling the gut microbiota, which then controls SINV replication. Further studies are necessary to clarify the role of the IMD pathway in antiviral defense. Specifically, attention needs to be payed to the clear distinction between the impact of the IMD pathway and the Janus kinase transducer and activator of transcription (JAK-STAT) pathway which both can be activated in mosquitoes by similar stimuli. The insects' JAK-STAT pathway was initially described as a response to stress in *Drosophila* but has been linked with antiviral response in the fly through a microarray study (Dostert et al. 2005). Further evidence of a functional involvement of JAK-STAT pathway in antiviral defense arose from infection experiments of flies with mutations in the Janus kinase gene *hopscotch* (*hop*) with a panel of viruses. These experiments showed that the JAK-STAT pathway is essential to control *Dicistroviridae* (e.g., *Drosophila C virus*) infection in *Drosophila* but is dispensable for antiviral immunity against other viruses tested (Kemp et al. 2013). Bioinformatic analysis of mosquito genome data showed that orthologues of JAK-STAT pathway components, namely, the *domeless* (*dome*) receptor, the *hop* kinase, and STAT transcription factor, are also found in *Anopheles gambiae* and *Aedes aegypti* mosquitoes (Souza-Neto et al. 2009; Waterhouse et al. 2007). Infection of *Aedes aegypti* mosquitoes with DENV significantly induces the JAK-STAT pathway, and silencing of *dome* or *hop* leads to increased virus replication (Souza-Neto et al. 2009; Xi et al. 2008). Furthermore, a recent study by Jupatanakul et al. (2017) demonstrated that genetically engineered mosquitoes overexpressing *dome* and *hop* in the fat body have significantly reduced DENV replication in their bodies and most importantly largely reduced DENV infection rates in the salivary glands. However, the infection rates of ZIKV and CHIKV were not affected in the same mosquitoes. In contrast, Angleró-Rodríguez et al. (2017) demonstrated that ZIKV modulates the expression of Toll-, IMD-, and JAK-STAT-associated genes in *Aedes aegypti* and that the activation of Toll and JAK-STAT pathway significantly reduces ZIKV replication. Thus, it is not clear whether the JAK-STAT pathway is a

pan-flavivirus-specific antiviral pathway similar to what was observed for *Dicistroviridae* in *Drosophila* or whether the antiviral function of this pathway is strictly virus species-specific. Furthermore, it is not clear if JAK-STAT pathway induction has a similar antiviral effect in other mosquito species. Data from WNV infection in *Culex quinquefasciatus* mosquitoes indicate that activation of the JAK-STAT pathway controls virus replication in these mosquitoes. Interestingly, the pathway is activated through secreted Vago, which is induced in a Dicer-2-dependent manner, thereby providing first evidence for a JAK-STAT-RNAi pathway cross talk (Paradkar et al. 2012). In contrast to *Aedes* and *Culex* mosquitoes, *Anopheles gambiae* mosquitoes do not show any transcriptional activation of JAK-STAT or Toll and IMD pathways after experimental infection with ONNV nor did a knockdown of components of this pathway impact ONNV replication (Waldock et al. 2012).

9.3.2 Virus Adaptation to the Mosquito: Immune Evasion and Immune Suppression by Arboviruses

Viruses are constantly exposed to the immune system of their hosts/vectors, which seeks to eliminate viral infection. In consequence, viral pathogens have evolved mechanisms to evade the immune system and infect new vectors.

Genetic reassortment is an important source of antigenic variability for segmented RNA viruses. It allows the fast antigenic shift instead of the slower antigenetic drift and, therefore, is one important factor for the evolution and emergence of viruses with an altered phenotype, disease potential, or host range (Gerrard et al. 2004; Kilian et al. 2013). Extinct or "new" viruses with greater pathogenicity might be created by natural or laboratory reassortment (Briese et al. 2013). An introduction of BUNV (*Orthobunyavirus*, *Peribunyaviridae*) or *La Crosse virus* (LACV) exotic to Europe and the possibility of reassortment with BATV (infraspecies belonging to the *Bunyamwera orthobunyavirus* species and serogroup), respectively, and TAHV (infraspecies belonging to the *California encephalitis orthobunyavirus* species and serogroup) endemic in Europe that may lead to reassortants with greater pathogenicity for humans or other vertebrates have to be considered (Briese et al. 2013; Eiden et al. 2014; Rudolf 2015). Bunyaviruses inherit a tripartite genome consisting of a small (S), medium (M), and large (L) segment. In Africa, NRIV and BUNV have similar geographic distributions across a broad region of sub-Saharan Africa, and both viruses have been isolated from the same species of *Aedes* mosquitoes (Gerrard et al. 2004). Importantly, a large outbreak of hemorrhagic fever in humans in East Africa in late 1997 and early 1998 was related to NRIV, which was found a reassortant of BUNV (S and L segment) and BATV (M segment) (Gerrard et al. 2004). Vector competence studies with *Culex quinquefasciatus*, *Anopheles gambiae*, and *Aedes aegypti* revealed considerable differences in their susceptibility to oral BUNV and NRIV infection. *Culex quinquefasciatus* was refractory and *Anopheles gambiae* moderately susceptible to both viruses. Interestingly, *Aedes aegypti* was moderately susceptible to BUNV but refractory to

NRIV infection (Odhiambo et al. 2014). Therefore, considerable differences in the host range of viruses within the same *Orthobunyavirus* serogroup may occur.

Reassortment is a major driver of rapid evolution in viruses, such as genetic reassortment of avian and human influenza A viruses, bunyaviruses, or bluetongue viruses. Bunyaviruses are considered to originate from strictly inter-mosquito-transmitted viruses, and evolution led to adaptation to vertebrate hosts (Junglen 2016). A marked number of orthobunyaviruses lack the open reading frame encoding nonstructural NSs protein. For example, a novel clade of mosquito-associated bunyaviruses (herbeviruses) and viruses belongs to the *Anopheles A*, *Anopheles B*, and *Tete* serogroups. The orthobunyaviruses that lack the NSs protein fail to prevent induction of interferon-beta mRNA in mammalian cells (Hollidge et al. 2011; Marklewitz et al. 2013; Mohamed et al. 2009; Shchetinin et al. 2015; Weber et al. 2002). Reports about whether NSs may have an effect on virus growth and RNAi in insect cells are controversial (Hart et al. 2009; Hollidge et al. 2011; Rudolf 2015). The phenotype of closely related virus strains of the same species may even differ in their route of transmission. An originally strictly inter-arthropod-transmitted virus circulating within arthropod populations may convert to an arthropod-borne virus (may be due to evolutionary advantages, faster temporal and spatial distribution, and therefore higher fitness) that is more efficiently transmitted using an intermediate amplifying host, e.g., vertebrate hosts. Furthermore, a change in phenotype from a strictly inter-arthropod-transmitted virus toward a virus that may be directly transmitted between mammals cannot be precluded, as previously described for the two atypical bluetongue virus (BTV) serotypes 26 and 27 (Batten et al. 2014; Bréard et al. 2018).

In nature, reassortment may occur in the vertebrate host or the mosquito vector. However, studies of genome reassortment of orthobunyaviruses in vertebrates were unsuccessful (Beaty et al. 1985). In contrast, reassortment of heterologous as well as homologous orthobunyaviruses (*Peribunyaviridae*, *Bunyavirales*) was demonstrated in the mosquito vector (Borucki et al. 1999). In *Aedes triseriatus* mosquitoes, 20% of the offspring transovarially infected with LACV became superinfected when challenged with a second LACV strain or the serologically closely related *Snowshoe hare virus* (SSHV) (Borucki et al. 1999). Furthermore, a greater viral intra-host diversity was detected in ticks infected with *Crimean-Congo hemorrhagic fever orthonairovirus* (CCHFV; another member of the *Bunyavirales* order) compared to the vertebrate host (Xia et al. 2016) suggesting the arthropod vectors are the primary source of antigenic shift and drift. Accordingly, there is an urgent need to continually monitor emergent arboviral genotypes circulating within particular regions as well as vectors mediating these transmissions to preempt and prevent their adverse effects, genetic mechanism for species specificity, and vector competence owing to reassortment that needs further investigation (Odhiambo et al. 2014).

Besides such drastic measures as exchange of genome segments, viruses have developed other mechanisms to avoid or circumvent the vector and host immune systems. A study by Brackney et al. (2009) showed that siRNAs generated from WNV genomes in *Culex* mosquitoes mostly matched specific "hot spot" regions in the virus genome. These specific regions were more prone to mutations than other so-called cold spots, indicating that an enhanced mutation rate is one mechanism to

escape siRNAs targeting. Also, abundantly expressed sub-genomic RNAs derived from the highly structured RNA encoded in the 3′ untranslated region of all flaviviruses (sub-genomic flavivirus (sf) RNAs) have been described (Pijlman et al. 2008). The sfRNAs derived from DENV and WNV genomes during infection have been shown to suppress Dcr-2-dependent dsRNA cleavage most likely through a direct inhibition of Dcr-2 (Moon et al. 2015; Schnettler et al. 2012). Furthermore, this inhibition in mosquito immunity by sfRNAs is curtail for successful virus transmission as shown by decreased transmission of sfRNA-deficient WNV in *Culex* mosquitoes and enhanced transmission of sfRNA overexpressing DENV in *Aedes aegypti* (Moon et al. 2015; Pompon et al. 2017).

Looking at classical viral suppressors of RNA silencing proteins (VSRs), diverse examples with multiple modes of action can be found. However, some common themes were established during evolution. The NSs protein of the plant-infecting *Tomato spotted wilt orthotospovirus* (TSWV; *Tospoviridae*, *Bunyavirales*), the B2 protein of the insect *Flock house virus* (FHV; *Nodaviridae*), and the VP3 protein of the mosquito-specific *Culex Y virus* (CYV; *Birnaviridae*) all sequester dsRNA molecules of different lengths and by this inhibit the recognition of these RNA molecules by Dcr-2 and incorporation of small dsRNA species into RISC, respectively. For arboviruses, not a lot of those classical VSRs have been identified; some authors even speculate that arboviruses do not express VSRs as matter of adaptation to avoid undue replication of the virus in the mosquito vector. Nevertheless, the DENV NS4B protein was shown to have VSR activity. While it could not bind to dsRNAs, it interferes with dicing (Kakumani et al. 2013).

9.3.3 Impact of **Wolbachia** on Vector Competence and Virus Replication

Several studies during recent years have shown that *Wolbachia*, a family of endosymbiotic *Alphaproteobacteria*, are associated with resistance to viral infection in several insect species including *Drosophila* (Hedges et al. 2008) and mosquito species (Glaser and Meola 2010; Moreira et al. 2009). *Wolbachia pipientis* was first described in the mosquito *Culex pipiens* and is inherited maternally via egg cytoplasm. The effects of *Wolbachia* infection in mosquitoes are widespread and somewhat contradictory. Several studies describe inhibitory effects on the infection with pathogens, especially in mosquitoes that have been artificially infected. However, others have reported no effect on virus infection or even enhanced virus infection rates (reviewed in Johnson (2015)).

Concerning the *Culex* complex, up to now, reported observations are contradictory. Glaser and Meola described an enhanced resistance of *Wolbachia*-infected *Culex quinquefasciatus* toward WNV (Glaser and Meola 2010), whereas Dodson et al. (2014) reported an enhancement of WNV replication in artificially *Wolbachia*-infected *Culex tarsalis* mosquitoes. Furthermore, the resistance phenotype in *Culex quinquefasciatus* as well as *Culex pipiens* toward WNV is not only dependent on

the presence or absence but also on the *Wolbachia* density (Micieli and Glaser 2014). In *Aedes* mosquitoes, repression of virus infection but again lack of effect on virus replication due to *Wolbachia* infection was observed. The most impressive phenotypes were observed in *Aedes aegypti*, which is one of the few mosquito species not naturally infected with *Wolbachia*. Artificial transfection of the *Drosophila* *wMel* and *wMelPop* strains leads to severe reduction of virus replication, dissemination, and transmission of DENV, YFV, CHIKV, and WNV (Hussain et al. 2013; Moreira et al. 2009; van den Hurk et al. 2012; Walker et al. 2011). The transinfection with the *wAlbB* strain from *Aedes albopictus* also reduced DENV infection (Bian et al. 2010). However, naturally *Wolbachia* (*wAlbA* and *wAlbB*)-infected *Aedes albopictus* did not show virus repression phenotypes when transinfected with the *Drosophila*-specific *Wolbachia* strain *wMel*. Only on rare occasions, i.e., when Blagrove et al. (2012) replaced the natural *Wolbachia* strain by the *wMel* strain, they were able to observe reduced transmission efficiency in those mosquitoes for DENV.

The mechanisms underlying the resistance phenotype are poorly understood. Some studies link the effect of *Wolbachia* infection in mosquitoes to immune priming. In *Drosophila* several studies argue against an involvement of Toll an IMD pathway priming, since Rancès et al. (2013) showed that priming of these pathways is not necessary for the *Wolbachia*-mediated blocking of DENV and Chrostek et al. (2014) found a high antiviral protection without immune upregulation after interspecies transfer of *Wolbachia.* Most studies analyzing the resistance phenotype in mosquitoes used *Aedes aegypti* mosquitoes and DENV infection. This combination has shown the most pronounced resistance phenotype, which might be due to the fact that *Aedes aegypti* mosquitoes are not naturally infected with *Wolbachia*. The activation of the Toll, IMD, JAK-STAT, and melanization pathways in *Aedes aegypti* was investigated among others by Kambris et al. (2009). Other studies have demonstrated the crucial role of the Toll pathway to control DENV infection in these mosquitoes (Xi et al. 2008). Hence, it is rational to suspect a causative link between the blocking phenotype and immune activation. Indeed, such a link could be established by Pan et al. (2012). Recent data collected from *wAlbB*-infected *Aedes aegypti* mosquitoes even show that the permanent activation of the Toll and IMD pathways by *Wolbachia* is needed by the bacteria to establish a stable infection in the mosquito (Pan et al. 2018). However, the same is not the case in the natural DENV vector *Aedes albopictus.* An additional transinfection with the heterologous *wMel* strain does not lead to significant upregulation of the innate immune pathways (Blagrove et al. 2012). A recent study with *Wolbachia*-infected *Aedes aegypti* cell lines demonstrated that the induction of RNAi, Toll, and IMD pathways must not necessarily be the cause of the protective effect since only the knockdown of the RNAi pathway leads to a small but significant reduction of the protective phenotype (Terradas et al. 2017). It has been demonstrated that a Vago homolog in *Aedes aegypti* (*AedesVago1*) inhibits the replication of DENV (Asad et al. 2017). This is of special interest, since Vago has been demonstrated to be induced in a Dcr-2-dependent manner in *Culex quinquefasciatus* and facilitated crosstalk between the exoRNAi pathway and the JAK-STAT pathways. Taken together, all data on immune priming and the inhibition phenotype induced by

Wolbachia show that immune priming might explain this effect in parts. However, other mechanisms need to be considered to fully explain the inhibition phenotype. A couple of studies create possible links between insect cell physiology and infection resistance. The infections of *Aedes aegypti* cells with the *Wolbachia* strain *wMelpop* leads to a downregulation of MCT1 expression (Osei-Amo et al. 2012). Since alteration of MCT expression has been shown to induce apoptosis in insects (Jang et al. 2008), there might be a link between enhanced apoptosis and reduced virus replication. Also the energy metabolism of cells is discussed as possible cause for virus inhibition. DENV is known to manipulate the cellular fatty acid biosynthetic pathway to create a favorable environment for viral replication complexes at intracellular membranes (Perera et al. 2012). It has been also shown that *Wolbachia* requires unsaturated fatty acids from host cells, since it cannot synthesize those. Thus, both bacteria and viruses need unsaturated fatty acid from the host; by limiting this resource, bacterial growth could suppress virus replication. Cholesterol was also shown to be crucial for DENV and alphavirus replication (Hafer et al. 2009; Lu et al. 1999; Rothwell et al. 2009) as well as for *Wolbachia* replication. A high growth rate of *Wolbachia* could deplete the insect cells from cholesterol and by this block the virus from essential resources for its replication (Moreira et al. 2009). This hypothesis was also confirmed in *Drosophila* when a cholesterol-enriched diet reduced the protective effect of *Wolbachia* against virus infection (Caragata et al. 2013). In *Aedes aegypti* cells, treatment with 2-hydroxypropyl-β-cyclodextrin to restore cholesterol homeostasis rescued DENV replication (Geoghegan et al. 2017). To confirm this mechanism in different mosquito and virus species, future studies will be necessary.

9.4 Conclusions

It can be summarized that virus replication in vector mosquitoes and thus the emergence of arbovirus infection are controlled by a myriad of different factors, including the mosquito abundance, temperature profiles, habitats, abundance of susceptible hosts, and other external factors. Also, the importance of intrinsic factors including the immune system of the insect and the presence of microbiota and endosymbionts such as *Wolbachia* cannot be stressed enough. The available data draw a complicated picture of the importance of all different factors which all warrant further research for clarification, altogether making vector competence studies a challenging but no less fascinating future topic.

References

ages (2017) https://www.ages.at/service/service-presse/pressemeldungen/west-nil-virus-situation-in-oesterreich/

Amraoui F, Failloux AB (2016) Chikungunya: an unexpected emergence in Europe. Curr Opin Virol 21:146–150. https://doi.org/10.1016/j.coviro.2016.09.014

Angel A, Angel B, Joshi V (2016) Rare occurrence of natural transovarial transmission of *Dengue virus* and elimination of infected foci as a possible intervention method. Acta Trop 155:20–24. https://doi.org/10.1016/j.actatropica.2015.11.018

Angenvoort J, Brault AC, Bowen RA, Groschup MH (2013) West Nile viral infection of equids. Vet Microbiol 167:168–180. https://doi.org/10.1016/j.vetmic.2013.08.013

Angleró-Rodríguez YI, MacLeod HJ, Kang S, Carlson JS, Jupatanakul N, Dimopoulos G (2017) *Aedes aegypti* Molecular responses to *Zika virus*: modulation of infection by the Toll and Jak/Stat Immune pathways and virus host factors. Front Microbiol 8:2050. https://doi.org/10.3389/fmicb.2017.02050

Arensburger P et al (2010) Sequencing of *Culex quinquefasciatus* establishes a platform for mosquito comparative genomics. Science 330:86–88. https://doi.org/10.1126/science.1191864

Asad S, Parry R, Asgari S (2017) Upregulation of *Aedes aegypti* Vago1 by *Wolbachia* and its effect on *Dengue virus* replication. Insect Biochem Mol Biol 92:45–52. https://doi.org/10.1016/j.ibmb.2017.11.008

Asgari S (2014) Role of microRNAs in arbovirus/vector interactions. Viruses 6:3514–3534. https://doi.org/10.3390/v6093514

Ashfaq M, Hebert PD, Mirza JH, Khan AM, Zafar Y, Mirza MS (2014) Analyzing mosquito (Diptera: *Culicidae*) diversity in Pakistan by DNA barcoding. PLoS One 9:e97268. https://doi.org/10.1371/journal.pone.0097268

Avadhanula V, Weasner BP, Hardy GG, Kumar JP, Hardy RW (2009) A novel system for the launch of alphavirus RNA synthesis reveals a role for the Imd pathway in arthropod antiviral response. PLoS Pathog 5:e1000582. https://doi.org/10.1371/journal.ppat.1000582

Bahnck CM, Fonseca DM (2006) Rapid assay to identify the two genetic forms of *Culex (Culex) pipiens* L. (Diptera: *Culicidae*) and hybrid populations. Am J Trop Med Hyg 75:251–255

Barletta ABF, Nascimento-Silva MCL, Talyuli OAC, Oliveira JHM, Pereira LOR, Oliveira PL, Sorgine MHF (2017) Microbiota activates IMD pathway and limits Sindbis infection in *Aedes aegypti*. Parasit Vectors 10:103. https://doi.org/10.1186/s13071-017-2040-9

Bartholomay LC et al (2010) Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens. Science 330:88–90. https://doi.org/10.1126/science.1193162

Batten C et al (2014) Evidence for transmission of *Bluetongue virus* serotype 26 through direct contact. PLoS One 9:e96049. https://doi.org/10.1371/journal.pone.0096049

Beaty BJ, Sundin DR, Chandler LJ, Bishop DH (1985) Evolution of bunyaviruses by genome reassortment in dually infected mosquitoes (*Aedes triseriatus*). Science 230:548–550

Becker N, Petric D, Zgomba M, Boase C, Madon MB, Dahl C, Kaiser A (2010) Mosquitoes and their control. Springer, Berlin. Hardcover ISBN 978-3-540-92873-7. https://doi.org/10.1007/978-3-540-92874-4

Bian G, Xu Y, Lu P, Xie Y, Xi Z (2010) The endosymbiotic bacterium Wolbachia induces resistance to *Dengue virus* in *Aedes aegypti*. PLoS Pathog 6:e1000833. https://doi.org/10.1371/journal.ppat.1000833

Bickford D et al (2007) Cryptic species as a window on diversity and conservation. Trends Ecol Evol 22:148–155. https://doi.org/10.1016/j.tree.2006.11.004

Blagrove MSC, Arias-Goeta C, Failloux A-B, Sinkins SP (2012) *Wolbachia* strain *wMel* induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. Proc Natl Acad Sci U S A 109:255–260. https://doi.org/10.1073/pnas.1112021108

Blagrove MS et al (2016) Evaluation of the vector competence of a native UK mosquito *Ochlerotatus detritus* (*Aedes detritus*) for *Dengue*, *Chikungunya* and *West Nile viruses*. Parasit Vectors 9:452. https://doi.org/10.1186/s13071-016-1739-3

Blair CD, Olson KE (2015) The role of RNA interference (RNAi) in arbovirus-vector interactions. Viruses 7:820–843. https://doi.org/10.3390/v7020820

Boccolini D et al (2016) Experimental investigation of the susceptibility of *Italian Culex pipiens* mosquitoes to *Zika virus* infection. Euro Surveill 21. https://doi.org/10.2807/1560-7917.ES.2016.21.35.30328

Bonilauri P et al (2008) *Chikungunya virus* in *Aedes albopictus*, Italy. Emerg Infect Dis 14:852–854. https://doi.org/10.3201/eid1405.071144

Borstler J et al (2014) The use of morphometric wing characters to discriminate female *Culex pipiens* and *Culex torrentium*. J Vector Ecol 39:204–212. https://doi.org/10.1111/j.1948-7134.2014.12088.x

Borucki MK, Chandler LJ, Parker BM, Blair CD, Beaty BJ (1999) Bunyavirus superinfection and segment reassortment in transovarially infected mosquitoes. J Gen Virol 80(Pt 12):3173–3179. https://doi.org/10.1099/0022-1317-80-12-3173

Boyle DB, Bulach DM, Amos-Ritchie R, Adams MM, Walker PJ, Weir R (2012) Genomic sequences of Australian *Bluetongue virus* prototype serotypes reveal global relationships and possible routes of entry into Australia. J Virol 86:6724–6731. https://doi.org/10.1128/JVI.00182-12

Boyle DB et al (2014) Evolution of *Bluetongue virus* serotype 1 in northern Australia over 30 years. J Virol 88:13981–13989. https://doi.org/10.1128/JVI.02055-14

Brackney DE, Beane JE, Ebel GD (2009) RNAi targeting of *West Nile virus* in mosquito mid-guts promotes virus diversification. PLoS Pathog 5:e1000502. https://doi.org/10.1371/journal.ppat.1000502

Brackney DE et al (2010) C6/36 *Aedes albopictus* cells have a dysfunctional antiviral RNA interference response. PLoS Negl Trop Dis 4:e856. https://doi.org/10.1371/journal.pntd.0000856

Bréard E et al (2018) *Bluetongue virus* serotype 27: experimental infection of goats, sheep and cattle with three BTV-27 variants reveal atypical characteristics and likely direct contact transmission BTV-27 between goats. Transbound Emerg Dis 65(2):e251–e263. https://doi.org/10.1111/tbed.12780

Brennecke J, Aravin AA, Stark A, Dus M, Kellis M, Sachidanandam R, Hannon GJ (2007) Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. Cell 128:1089–1103. https://doi.org/10.1016/j.cell.2007.01.043

Briese T, Calisher CH, Higgs S (2013) Viruses of the family Bunyaviridae: are all available isolates reassortants? Virology 446:207–216. https://doi.org/10.1016/j.virol.2013.07.030

Brustolin M et al (2016) *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*) populations as vectors for lineage 1 and 2 *West Nile virus* in Europe. Med Vet Entomol 30:166–173. https://doi.org/10.1111/mve.12164

Brustolin M et al (2017) *Rift Valley fever virus* and European mosquitoes: vector competence of *Culex pipiens* and *Stegomyia albopicta* (= *Aedes albopictus*). Med Vet Entomol 31:365–372. https://doi.org/10.1111/mve.12254

Bunning ML et al (2002) Experimental Infection of horses with *West Nile virus*. Emerg Infect Dis 8:380–386. https://doi.org/10.3201/eid0804.010239

Calzolari M et al (2012) Mosquito, bird and human surveillance of *West Nile* and *Usutu viruses* in Emilia-Romagna Region (Italy) in 2010. PLoS One 7:e38058. https://doi.org/10.1371/journal.pone.0038058

Calzolari M, Bonilauri P, Bellini R, Becker S, Dottori M (2016) Wide recognition of *Culex pipiens* and lack of detection of *Culex torrentium* through biomolecular differentiation of mosquitoes in the Emilia-Romagna region, Northern Italy. Med Vet Entomol 30:435–438. https://doi.org/10.1111/mve.12186

Campbell CL, Black WC 4th, Hess AM, Foy BD (2008a) Comparative genomics of small RNA regulatory pathway components in vector mosquitoes. BMC Genomics 9:425. https://doi.org/10.1186/1471-2164-9-425

Campbell CL, Keene KM, Brackney DE, Olson KE, Blair CD, Wilusz J, Foy BD (2008b) *Aedes aegypti* uses RNA interference in defense against *Sindbis virus* infection. BMC Microbiol 8:47. https://doi.org/10.1186/1471-2180-8-47

Campbell CL, Harrison T, Hess AM, Ebel GD (2014) MicroRNA levels are modulated in *Aedes aegypti* after exposure to Dengue-2. Insect Mol Biol 23:132–139. https://doi.org/10.1111/imb.12070

Caragata EP, Rancès E, Hedges LM, Gofton AW, Johnson KN, O'Neill SL, McGraw EA (2013) Dietary cholesterol modulates pathogen blocking by *Wolbachia*. PLoS Pathog 9:e1003459. https://doi.org/10.1371/journal.ppat.1003459

Carissimo G et al (2015) Antiviral immunity of Anopheles gambiae is highly compartmentalized, with distinct roles for RNA interference and gut microbiota. Proc Natl Acad Sci U S A 112:E176–E185. https://doi.org/10.1073/pnas.1412984112

Carpenter S, Groschup M, Garros C, Felippe-Bauer ML, Purse B (2013) *Culicoides* biting midges, arboviruses and public health in Europe. Antiviral Res 100(1):102–113. https://doi.org/10.1016/j.antiviral.2013.07.020

CDC (2017) https://www.cdc.gov/chikungunya/geo/united-states-2017.html

Chotkowski HL, Ciota AT, Jia Y, Puig-Basagoiti F, Kramer LD, Shi PY, Glaser RL (2008) *West Nile virus* infection of *Drosophila melanogaster* induces a protective RNAi response. Virology 377:197–206. https://doi.org/10.1016/j.virol.2008.04.021

Chrostek E, Marialva MSP, Yamada R, O'Neill SL, Teixeira L (2014) High anti-viral protection without immune upregulation after interspecies *Wolbachia* transfer. PLoS One 9:e99025. https://doi.org/10.1371/journal.pone.0099025

Cirimotich CM, Scott JC, Phillips AT, Geiss BJ, Olson KE (2009) Suppression of RNA interference increases alphavirus replication and virus-associated mortality in *Aedes aegypti* mosquitoes. BMC Microbiol 9:49. https://doi.org/10.1186/1471-2180-9-49

Cleton N, Koopmans M, Braks M, Van Maanen K, Reusken C (2014) [Japanese encephalitis in Southern Europe]. Tijdschr Diergeneeskd 139:20–25

Colpitts TM et al (2011) Alterations in the *Aedes aegypti* transcriptome during infection with *West Nile*, *Dengue* and *Yellow fever viruses*. PLoS Pathog 7:e1002189. https://doi.org/10.1371/journal.ppat.1002189

Costa A, Jan E, Sarnow P, Schneider D (2009) The Imd pathway is involved in antiviral immune responses in *Drosophila*. PLoS One 4:e7436. https://doi.org/10.1371/journal.pone.0007436

Crabtree MB, Savage HM, Miller BR (1995) Development of a species-diagnostic polymerase chain reaction assay for the identification of Culex vectors of *St. Louis encephalitis virus* based on interspecies sequence variation in ribosomal DNA spacers. Am J Trop Med Hyg 53: 105–109

de Wispelaere M, Despres P, Choumet V (2017) European *Aedes albopictus* and *Culex pipiens* are competent vectors for *Japanese encephalitis virus*. PLoS Negl Trop Dis 11:e0005294. https://doi.org/10.1371/journal.pntd.0005294

Dejnirattisai W et al (2010) Cross-reacting antibodies enhance *Dengue virus* infection in humans. Science 328:745–748. https://doi.org/10.1126/science.1185181

Delisle E et al (2015) Chikungunya outbreak in Montpellier, France, September to October 2014. Euro Surveill 20

Di Luca M et al (2016) Experimental studies of susceptibility of Italian *Aedes albopictus* to *Zika virus*. Euro Surveill 21. https://doi.org/10.2807/1560-7917.ES.2016.21.18.30223

Dierig A, Frei R, Egli A (2015) The fast route to microbe identification: matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). Pediatr Infect Dis J34:97–99. https://doi.org/10.1097/INF.0000000000000601

Dietrich I et al (2017a) RNA interference restricts *Rift Valley fever virus* in multiple insect systems. mSphere 2

Dietrich I et al (2017b) The antiviral RNAi response in vector and non-vector cells against orthobunyaviruses. PLoS Negl Trop Dis 11:e0005272. https://doi.org/10.1371/journal.pntd.0005272

Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD, Rasgon JL (2014) *Wolbachia* enhances *West Nile virus* (WNV) Infection in the Mosquito *Culex tarsalis*. PLoS Negl Trop Dis 8:e2965. https://doi.org/10.1371/journal.pntd.0002965

Dohm DJ, Logan TM, Barth JF, Turell MJ (1995) Laboratory transmission of *Sindbis virus* by *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens* (Diptera: Culicidae). J Med Entomol 32:818–821

Donald CL, Kohl A, Schnettler E (2012) New insights into control of arbovirus replication and spread by insect RNA interference pathways. Insects 3:511–531. https://doi.org/10.3390/insects3020511

Dong Y, Manfredini F, Dimopoulos G (2009) Implication of the mosquito midgut microbiota in the defense against malaria parasites. PLoS Pathog 5:e1000423. https://doi.org/10.1371/journal.ppat.1000423

Dostert C et al (2005) The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *drosophila*. Nat Immunol 6:946–953. https://doi.org/10.1038/ni1237

Eckerle I et al (2018) Emerging souvenirs—clinical presentation of the returning traveller with imported arbovirus infections in Europe. Clin Microbiol Infect 24(3):240–245. https://doi.org/10.1016/j.cmi.2018.01.007

Eiden M et al (2014) *Ngari virus* in goats during Rift Valley fever outbreak, Mauritania, 2010. Emerg Infect Dis 20:2174–2176. https://doi.org/10.3201/eid2012.140787

Engdahl C et al (2014) Identification of Swedish mosquitoes based on molecular barcoding of the COI gene and SNP analysis. Mol Ecol Resour 14:478–488. https://doi.org/10.1111/1755-0998.12202

Feltens R, Gorner R, Kalkhof S, Groger-Arndt H, von Bergen M (2010) Discrimination of different species from the genus *Drosophila* by intact protein profiling using matrix-assisted laser desorption ionization mass spectrometry. BMC Evol Biol 10:95. https://doi.org/10.1186/1471-2148-10-95

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Fonseca D (2016) Approaches to Infer local vectorial capacity: from rapid assays to population genomics and transcriptomics. Nova Acta Leopold 411:11–21

Fonseca DM et al (2004) Emerging vectors in the *Culex pipiens* complex. Science 303:1535–1538. https://doi.org/10.1126/science.1094247

Fortuna C et al (2015a) Experimental studies on comparison of the vector competence of four Italian *Culex pipiens* populations for *West Nile virus*. Parasit Vectors 8:463. https://doi.org/10.1186/s13071-015-1067-z

Fortuna C et al (2015b) Evaluation of vector competence for *West Nile virus* in Italian *Stegomyia albopicta* (=*Aedes albopictus*) mosquitoes. Med Vet Entomol 29:430–433. https://doi.org/10.1111/mve.12133

Fros JJ et al (2015a) *West Nile virus*: high transmission rate in North-Western European mosquitoes indicates its epidemic potential and warrants increased surveillance. PLoS Negl Trop Dis 9:e0003956. https://doi.org/10.1371/journal.pntd.0003956

Fros JJ et al (2015b) Comparative *Usutu and West Nile virus* transmission potential by local *Culex pipiens* mosquitoes in north-western Europe. One Health 1:31–36. https://doi.org/10.1016/j.onehlt.2015.08.002

Fu H, Leake CJ, Mertens PP, Mellor PS (1999) The barriers to *Bluetongue virus* infection, dissemination and transmission in the vector, *Culicoides variipennis* (Diptera: *Ceratopogonidae*). Arch Virol 144:747–761

Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL (2006) Essential function in vivo for Dicer-2 in host defense against RNA viruses in *drosophila*. Nat Immunol 7:590–597. https://doi.org/10.1038/ni1335

Gardner L, Chen N, Sarkar S (2017) Vector status of *Aedes* species determines geographical risk of autochthonous *Zika virus* establishment. PLoS Negl Trop Dis 11(3):e0005487. https://doi.org/10.1371/journal.pntd.0005487. Shepard DS (ed)

Garver LS, Bahia AC, Das S, Souza-Neto JA, Shiao J, Dong Y, Dimopoulos G (2012) *Anopheles* Imd pathway factors and effectors in infection intensity-dependent anti-Plasmodium action. PLoS Pathog 8:e1002737. https://doi.org/10.1371/journal.ppat.1002737

Geoghegan V et al (2017) Perturbed cholesterol and vesicular trafficking associated with dengue blocking in *Wolbachia*-infected *Aedes aegypti* cells. Nat Commun 8:526. https://doi.org/10.1038/s41467-017-00610-8

Gerrard SR, Li L, Barrett AD, Nichol ST (2004) *Ngari virus* is a *Bunyamwera virus* reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. J Virol 78:8922–8926. https://doi.org/10.1128/JVI.78.16.8922-8926.2004

Girard YA et al (2007) Salivary gland morphology and virus transmission during long-term cyto-pathologic *West Nile virus* infection in *Culex* mosquitoes. Am J Trop Med Hyg 76:118–128

Glaser RL, Meola MA (2010) The native *Wolbachia* endosymbionts of *Drosophila melanogaster* and *Culex quinquefasciatus* increase host resistance to *West Nile virus* infection. PLoS One 5:e11977. https://doi.org/10.1371/journal.pone.0011977

Gossner CM et al (2017) *West Nile virus* surveillance in Europe: moving towards an integrated animal-human-vector approach. Euro Surveill 22. https://doi.org/10.2807/1560-7917. ES.2017.22.18.30526

Gould EA, Gallian P, De Lamballerie X, Charrel RN (2010) First cases of autochthonous den-gue fever and chikungunya fever in France: from bad dream to reality! Clin Microbiol Infect 16:1702–1704. https://doi.org/10.1111/j.1469-0691.2010.03386.x

Guedes DR et al (2017) *Zika virus* replication in the mosquito *Culex quinquefasciatus* in Brazil. Emerg Microbes Infect 6:e69. https://doi.org/10.1038/emi.2017.59

Gunawardane LS et al (2007) A slicer-mediated mechanism for repeat-associated siRNA 5′ end formation in *Drosophila*. Science 315:1587–1590. https://doi.org/10.1126/science.1140494

Gutiérrez-Bugallo G et al (2017) First record of natural vertical transmission of *Dengue virus* in *Aedes aegypti* from Cuba. Acta Trop 174:146–148. https://doi.org/10.1016/j.actatropica.2017.07.012

Hafer A, Whittlesey R, Brown DT, Hernandez R (2009) Differential incorporation of choles-terol by *Sindbis virus* grown in mammalian or insect cells. J Virol 83:9113–9121. https://doi.org/10.1128/JVI.00755-09

Harbach RE (2004) The classification of genus Anopheles (Diptera: Culicidae): a working hypoth-esis of phylogenetic relationships. Bull Entomol Res 94:537–553

Hardy JL, Houk EJ, Kramer LD, Reeves WC (1983) Intrinsic factors affecting vector compe-tence of mosquitoes for arboviruses. Annu Rev Entomol 28:229–262. https://doi.org/10.1146/annurev.en.28.010183.001305

Hart TJ, Kohl A, Elliott RM (2009) Role of the NSs protein in the zoonotic capac-ity of Orthobunyaviruses. Zoonoses Public Health 56:285–296. https://doi.org/10.1111/j.1863-2378.2008.01166.x

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL (2005) Epidemiology and transmission dynamics of *West Nile virus* disease. Emerg Infect Dis 11:1167–1173. https://doi.org/10.3201/eid1108.050289a

Hebert PD, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proc Biol Sci 270:313–321. https://doi.org/10.1098/rspb.2002.2218

Hedges LM, Brownlie JC, O'Neill SL, Johnson KN (2008) *Wolbachia* and virus protection in insects. Science 322:702–702. https://doi.org/10.1126/science.1162418

Heitmann A et al (2017) Experimental transmission of *Zika virus* by mosquitoes from central Europe. Euro Surveill 22:30437. https://doi.org/10.2807/1560-7917.ES.2017.22.2.30437

Hess AM et al (2011) Small RNA profiling of *Dengue virus*-mosquito interactions impli-cates the PIWI RNA pathway in anti-viral defense. BMC Microbiol 11:45. https://doi.org/10.1186/1471-2180-11-45

Hesson JC, Lundstrom JO, Halvarsson P, Erixon P, Collado A (2010) A sensitive and reliable restriction enzyme assay to distinguish between the mosquitoes *Culex torrentium* and *Culex pipiens*. Med Vet Entomol 24:142–149. https://doi.org/10.1111/j.1365-2915.2010.00871.x

Hesson JC et al (2014) The arbovirus vector *Culex torrentium* is more prevalent than *Culex pipi-ens* in northern and central Europe. Med Vet Entomol 28:179–186. https://doi.org/10.1111/mve.12024

Hollidge BS, Weiss SR, Soldan SS (2011) The role of interferon antagonist, non-structural proteins in the pathogenesis and emergence of arboviruses. Viruses 3:629–658. https://doi.org/10.3390/v3060629

Holt RA et al (2002) The genome sequence of the malaria mosquito *Anopheles gambiae*. Science 298:129–149. https://doi.org/10.1126/science.1076181

Hubalek Z (2008) Mosquito-borne viruses in Europe. J Parasitol Res 103(Suppl 1):S29–S43. https://doi.org/10.1007/s00436-008-1064-7

Huber K et al (2014a) *Aedes japonicus japonicus* (Diptera: Culicidae) from Germany have vector competence for *Japan encephalitis virus* but are refractory to infection with *West Nile virus*. J Parasitol Res 113:3195–3199. https://doi.org/10.1007/s00436-014-3983-9

Huber K et al (2014b) Distribution and genetic structure of *Aedes japonicus japonicus* populations (Diptera: Culicidae) in Germany. J Parasitol Res 113:3201–3210. https://doi.org/10.1007/s00436-014-4000-z

Hussain M, Lu G, Torres S, Edmonds JH, Kay BH, Khromykh AA, Asgari S (2013) Effect of *Wolbachia* on replication of *West Nile virus* in a mosquito cell line and adult mosquitoes. J Virol 87:851–858. https://doi.org/10.1128/JVI.01837-12

Jang C, Lee G, Chung J (2008) LKB1 induces apical trafficking of Silnoon, a monocarboxylate transporter, in *Drosophila melanogaster*. J Cell Biol 183:11–17. https://doi.org/10.1083/jcb.200807052

Johnson K (2015) The impact of *Wolbachia* on virus infection in mosquitoes. Viruses 7:2903

Jost H, Bialonski A, Storch V, Gunther S, Becker N, Schmidt-Chanasit J (2010) Isolation and phylogenetic analysis of *Sindbis viruses* from mosquitoes in Germany. J Clin Microbiol 48:1900–1903. https://doi.org/10.1128/JCM.00037-10

Jost H et al (2011a) Isolation of *Usutu virus* in Germany. Am J Trop Med Hyg 85:551–553. https://doi.org/10.4269/ajtmh.2011.11-0248

Jost H, Bialonski A, Schmetz C, Gunther S, Becker N, Schmidt-Chanasit J (2011b) Isolation and phylogenetic analysis of *Batai virus*, Germany. Am J Trop Med Hyg 84:241–243. https://doi.org/10.4269/ajtmh.2011.10-0483

Junglen S (2016) Evolutionary origin of pathogenic arthropod-borne viruses—a case study in the family Bunyaviridae. Curr Opin Insect Sci 16:81–86. https://doi.org/10.1016/j.cois.2016.05.017

Jupatanakul N et al (2017) Engineered *Aedes aegypti* JAK/STAT pathway-mediated immunity to *Dengue virus*. PLoS Negl Trop Dis 11:e0005187. https://doi.org/10.1371/journal.pntd.0005187

Jupille H, Seixas G, Mousson L, Sousa CA, Failloux AB (2016) *Zika virus*, a new threat for Europe? PLoS Negl Trop Dis 10:e0004901. https://doi.org/10.1371/journal.pntd.0004901

Kakumani PK et al (2013) Role of RNA interference (RNAi) in *Dengue virus* replication and identification of NS4B as an RNAi suppressor. J Virol 87:8870–8883. https://doi.org/10.1128/JVI.02774-12

Kambris Z, Cook PE, Phuc HK, Sinkins SP (2009) Immune activation by life-shortening *Wolbachia* and reduced filarial competence in mosquitoes. Science (New York NY) 326:134–136. https://doi.org/10.1126/science.1177531

Kampen H, Werner D (2014) Out of the bush: the Asian bush mosquito *Aedes japonicus japonicus* (Theobald, 1901) (Diptera, Culicidae) becomes invasive. Parasit Vectors 7:59. https://doi.org/10.1186/1756-3305-7-59

Kampen H, Werner D (2015) [The recurring necessity of mosquito surveillance and research]. Bundesgesundheitsblatt, Gesundheitsforschung, Gesundheitsschutz 58:1101–1109

Kampen H, Kuhlisch C, Fröhlich A, Scheuch DE, Walther D (2016a) Occurrence and spread of the invasive Asian bush mosquito *Aedes japonicus japonicus* (Diptera: *Culicidae*) in West and North Germany since Detection in 2012 and 2013, Respectively. PLoS One 11:e0167948. https://doi.org/10.1371/journal.pone.0167948

Kampen H, Schäfer M, Zielke DE, Walther D (2016b) The *Anopheles maculipennis* complex (Diptera: *Culicidae*) in Germany: an update following recent monitoring activities. J Parasitol Res 115:3281–3294. https://doi.org/10.1007/s00436-016-5189-9

Kaufmann C, Schaffner F, Ziegler D, Pfluger V, Mathis A (2012) Identification of field-caught *Culicoides* biting midges using matrix-assisted laser desorption/ionization time of flight mass spectrometry. Parasitology 139:248–258. https://doi.org/10.1017/S0031182011001764

Keene KM, Foy BD, Sanchez-Vargas I, Beaty BJ, Blair CD, Olson KE (2004) RNA interference acts as a natural antiviral response *to O'nyong-nyong virus* (Alphavirus; *Togaviridae*) infection of *Anopheles gambiae*. Proc Natl Acad Sci U S A 101:17240–17245. https://doi.org/10.1073/pnas.0406983101

Kemp C et al (2013) Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in *Drosophila*. J Immunol 190:650–658. https://doi.org/10.4049/jimmunol.1102486

Khoo CC, Piper J, Sanchez-Vargas I, Olson KE, Franz AW (2010) The RNA interference pathway affects midgut infection- and escape barriers for *Sindbis virus* in *Aedes aegypti*. BMC Microbiol 10:130. https://doi.org/10.1186/1471-2180-10-130

Kilian P, Valdes JJ, Lecina-Casas D, Chrudimsky T, Ruzek D (2013) The variability of the large genomic segment of Tahyna orthobunyavirus and an all-atom exploration of its anti-viral drug resistance. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 20:304–311. https://doi.org/10.1016/j.meegid.2013.09.023

Kramer LD (2016) The impact of biotic and abiotic factors on vectorial capacity of *Culex mosquites* for WNV. Nova Acta Leopold 411:101–108

Kronefeld M, Dittmann M, Zielke D, Werner D, Kampen H (2012) Molecular confirmation of the occurrence in Germany of *Anopheles daciae* (Diptera, *Culicidae*). Parasit Vectors 5:250. https://doi.org/10.1186/1756-3305-5-250

Kronefeld M, Werner D, Kampen H (2014) PCR identification and distribution of *Anopheles daciae* (Diptera, *Culicidae*) in Germany. J Parasitol Res 113:2079–2086. https://doi.org/10.1007/s00436-014-3857-1

Lambrechts L (2010) Dissecting the genetic architecture of host–pathogen specificity. PLoS Pathog 6:e1001019. https://doi.org/10.1371/journal.ppat.1001019

Lambrechts L, Chevillon C, Albright RG, Thaisomboonsuk B, Richardson JH, Jarman RG, Scott TW (2009a) Genetic specificity and potential for local adaptation between *Dengue viruses* and mosquito vectors. BMC Evol Biol 9:160. https://doi.org/10.1186/1471-2148-9-160

Lambrechts L, Knox TB, Wong J, Liebman KA, Albright RG, Stoddard ST (2009b) Shifting priorities in vector biology to improve control of vector-borne disease. Trop Med Int Health 14:1505–1514. https://doi.org/10.1111/j.1365-3156.2009.02401.x

Leger P et al (2013) Dicer-2- and Piwi-mediated RNA interference in *Rift Valley fever virus*-infected mosquito cells. J Virol 87:1631–1648. https://doi.org/10.1128/JVI.02795-12

Li S, Mead EA, Liang S, Tu Z (2009) Direct sequencing and expression analysis of a large number of miRNAs in *Aedes aegypti* and a multi-species survey of novel mosquito miRNAs. BMC Genomics 10:581. https://doi.org/10.1186/1471-2164-10-581

Li C-x et al (2017) Vector competence and transovarial transmission of two *Aedes aegypti* strains to *Zika virus*. Emerg Microbes Infect 6:e23. https://doi.org/10.1038/emi.2017.8

Lim SM, Koraka P, von Boheemen S, Roose JM, Jaarsma D, von der Vijver DAMC, Osterhaus ADME, Byron EEM (2013) Characterization of the mouse neuroinvasiveness of selected European strains of *West Nile virus*. PLoS One 8:e74575. https://doi.org/10.1371/journal.pone.0074575

Linthicum KJ, Davies FG, Kairo A, Bailey CL (1985) *Rift Valley fever virus* (family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. J Hyg 95:197–209

Liu X, Jiang F, Kalidas S, Smith D, Liu Q (2006) Dicer-2 and R2D2 coordinately bind siRNA to promote assembly of the siRISC complexes. RNA 12:1514–1520. https://doi.org/10.1261/rna.101606

Lorenz C, Marques TC, Sallum MAM, Suesdek L (2012) Morphometrical diagnosis of the malaria vectors *Anopheles cruzii*, *An. homunculus* and *An. Bellator*. Parasit Vectors 5:257. https://doi.org/10.1186/1756-3305-5-257

Lorenz C et al (2017) Geometric morphometrics in mosquitoes: what has been measured? Infect Genet Evol 54:205–215. https://doi.org/10.1016/j.meegid.2017.06.029

Lourenço-de-Oliveira R, Rua AV, Vezzani D, Willat G, Vazeille M, Mousson L, Failloux AB (2013) *Aedes aegypti* from temperate regions of South America are highly competent to transmit *Dengue virus*. BMC Infect Dis 13:610–610. https://doi.org/10.1186/1471-2334-13-610

Lu YE, Cassese T, Kielian M (1999) The cholesterol requirement for *Sindbis virus* entry and exit and characterization of a spike protein region involved in cholesterol dependence. J Virol 73:4272–4278

Luhken R et al (2016) Distribution of individual members of the mosquito Anopheles maculipennis complex in Germany identified by newly developed real-time PCR assays. Med Vet Entomol 30:144–154. https://doi.org/10.1111/mve.12161

Luplertlop N et al (2011) Induction of a peptide with activity against a broad spectrum of pathogens in the *Aedes aegypti* salivary gland, following Infection with *Dengue virus*. PLoS Pathog 7:e1001252. https://doi.org/10.1371/journal.ppat.1001252

Mackenzie-Impoinvil L et al (2015) Evaluation of a temperate climate mosquito, *Ochlerotatus detritus* (=*Aedes detritus*), as a potential vector of *Japanese encephalitis virus*. Med Vet Entomol 29:1–9. https://doi.org/10.1111/mve.12083

Maclachlan NJ, Drew CP, Darpel KE, Worwa G (2009) The pathology and pathogenesis of bluetongue. J Comp Pathol 141:1–16. https://doi.org/10.1016/j.jcpa.2009.04.003

Mancini G et al (2017) Mosquito species involved in the circulation of *West Nile* and *Usutu viruses* in Italy. Vet Ital 53:97–110. https://doi.org/10.12834/VetIt.114.933.4764.2

Manni M et al (2017) Genetic evidence for a worldwide chaotic dispersion pattern of the arbovirus vector, *Aedes albopictus*. PLoS Negl Trop Dis 11:e0005332. https://doi.org/10.1371/journal.pntd.0005332

Marklewitz M et al (2013) Discovery of a unique novel clade of mosquito-associated bunyaviruses. J Virol 87:12850–12865. https://doi.org/10.1128/JVI.01862-13

Mathis A et al (2015) Identification of phlebotomine sand flies using one MALDI-TOF MS reference database and two mass spectrometer systems. Parasit Vectors 8:266. https://doi.org/10.1186/s13071-015-0878-2

Meister S et al (2005) Immune signaling pathways regulating bacterial and malaria parasite infection of the mosquito *Anopheles gambiae*. Proc Natl Acad Sci U S A 102:11420–11425. https://doi.org/10.1073/pnas.0504950102

Mellor P (2009) *Bluetongue virus* in the insect host. In: Mellor PS, Baylis M, Mertens PPC (eds) Bluetongue. Elsevier Academic Press, London, pp 295–320

Mellor PS, Boorman J, Baylis M (2000) *Culicoides* biting midges: their role as arbovirus vectors. Annu Rev Entomol 45:307–340. https://doi.org/10.1146/annurev.ento.45.1.307

Micieli MV, Glaser RL (2014) Somatic *Wolbachia* (Rickettsiales: *Rickettsiaceae*) levels in *Culex quinquefasciatus* and *Culex pipiens* (Diptera: *Culicidae*) and resistance to *West Nile virus* infection. J Med Entomol 51:189–199

Miesen P, Girardi E, van Rij RP (2015) Distinct sets of PIWI proteins produce arbovirus and transposon-derived piRNAs in *Aedes aegypti* mosquito cells. Nucleic Acids Res 43:6545–6556. https://doi.org/10.1093/nar/gkv590

Miesen P, Joosten J, van Rij RP (2016) PIWIs go viral: arbovirus-derived piRNAs in vector mosquitoes. PLoS Pathog 12:e1006017. https://doi.org/10.1371/journal.ppat.1006017

Mishra AC, Mourya DT (2001) Transovarial transmission of *West Nile virus* in *Culex vishnui* mosquito. Indian J Med Res 114:212–214

Mohamed M, McLees A, Elliott RM (2009) Viruses in the *Anopheles* A, *Anopheles* B, and Tete serogroups in the Orthobunyavirus genus (family *Bunyaviridae*) do not encode an NSs protein. J Virol 83:7612–7618. https://doi.org/10.1128/JVI.02080-08

Mohrig W (1969) Die Culiciden Deutschlands. Untersuchungen zur Taxonomie, Biologie und Ökologie der einheimischen Stechmücken, vol 18. Parasitologische Schriftenreihe

Moon SL, Dodd BJ, Brackney DE, Wilusz CJ, Ebel GD, Wilusz J (2015) Flavivirus sfRNA suppresses antiviral RNA interference in cultured cells and mosquitoes and directly interacts with the RNAi machinery. Virology 485:322–329. https://doi.org/10.1016/j.virol.2015.08.009

Morazzani EM, Wiley MR, Murreddu MG, Adelman ZN, Myles KM (2012) Production of virus-derived ping-pong-dependent piRNA-like small RNAs in the mosquito soma. PLoS Pathog 8:e1002470. https://doi.org/10.1371/journal.ppat.1002470

Moreira LA et al (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. Cell 139:1268–1278. https://doi.org/10.1016/j.cell.2009.11.042

Moutailler S, Krida G, Schaffner F, Vazeille M, Failloux AB (2008) Potential vectors of *Rift Valley fever virus* in the Mediterranean region. Vector Borne Zoonotic Dis 8:749–753. https://doi.org/10.1089/vbz.2008.0009

Moutailler S, Barre H, Vazeille M, Failloux AB (2009) Recently introduced *Aedes albopictus* in Corsica is competent to *Chikungunya virus* and in a lesser extent to *Dengue virus*. Trop Med Int Health 14:1105–1109. https://doi.org/10.1111/j.1365-3156.2009.02320.x

Mueller S et al (2010) RNAi-mediated immunity provides strong protection against the negative-strand RNA *Vesicular stomatitis virus* in *Drosophila*. Proc Natl Acad Sci U S A 107:19390–19395. https://doi.org/10.1073/pnas.1014378107

Mukherjee S, Hanley KA (2010) RNA interference modulates replication of *Dengue virus* in *Drosophila melanogaster* cells. BMC Microbiol 10:127. https://doi.org/10.1186/1471-2180-10-127

Muller S, Imler JL (2007) Dicing with viruses: microRNAs as antiviral factors. Immunity 27:1–3. https://doi.org/10.1016/j.immuni.2007.07.003

Mussabekova A, Daeffler L, Imler JL (2017) Innate and intrinsic antiviral immunity in *Drosophila*. Cell Mol Life Sci 74:2039–2054. https://doi.org/10.1007/s00018-017-2453-9

Myles KM, Wiley MR, Morazzani EM, Adelman ZN (2008) Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes. Proc Natl Acad Sci U S A 105:19938–19943. https://doi.org/10.1073/pnas.0803408105

Natvig H (1948) Contributions to the knowledge of the Danish and Fennoscandian mosquitoes. Norsk Ent Tidsskr Suppl 1:1–567

Negredo A et al (2017) Autochthonous Crimean-Congo hemorrhagic fever in Spain. N Engl J Med 377:154–161. https://doi.org/10.1056/NEJMoa1615162

Nene V et al (2007) Genome sequence of *Aedes aegypti*, a major arbovirus vector. Science 316:1718–1723. https://doi.org/10.1126/science.1138878

Nicolescu G, Linton YM, Vladimirescu A, Howard TM, Harbach RE (2004) Mosquitoes of the *Anopheles maculipennis* group (Diptera: Culicidae) in Romania, with the discovery and formal recognition of a new species based on molecular and morphological evidence. Bull Entomol Res 94:525–535

Nikolay B (2015) A review of *West Nile* and *Usutu virus* co-circulation in Europe: how much do transmission cycles overlap? Trans R Soc Trop Med Hyg 109:609–618. https://doi.org/10.1093/trstmh/trv066

Novikov YM, Shevchenko AI (2001) Inversion polymorphism and the divergence of two cryptic forms of *Anopheles messeae* (Diptera, *Culicidae*) at the level of genomic DNA repeats. Russ J Genet 37:754–763. https://doi.org/10.1023/a:1016790724790

Odhiambo C, Venter M, Chepkorir E, Mbaika S, Lutomiah J, Swanepoel R, Sang R (2014) Vector competence of selected mosquito species in Kenya for *Ngari* and *Bunyamwera viruses*. J Med Entomol 51:1248–1253. https://doi.org/10.1603/ME14063

Osei-Amo S, Hussain M, O'Neill SL, Asgari S (2012) *Wolbachia*-induced aae-miR-12 miRNA negatively regulates the expression of MCT1 and MCM6 genes in *Wolbachia*-infected mosquito cell line. PLoS One 7:e50049. https://doi.org/10.1371/journal.pone.0050049

Pan X, Zhou G, Wu J, Bian G, Lu P, Raikhel AS, Xi Z (2012) *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control *Dengue virus* in the mosquito *Aedes aegypti*. Proc Natl Acad Sci U S A 109:E23–E31. https://doi.org/10.1073/pnas.1116932108

Pan X et al (2018) The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti*. ISME J 12:277–288. https://doi.org/10.1038/ismej.2017.174

Paradkar PN, Trinidad L, Voysey R, Duchemin JB, Walker PJ (2012) Secreted Vago restricts *West Nile virus* infection in *Culex* mosquito cells by activating the Jak-STAT pathway. Proc Natl Acad Sci U S A 109:18915–18920. https://doi.org/10.1073/pnas.1205231109

Perera R et al (2012) *Dengue virus* infection perturbs lipid homeostasis in infected mosquito cells. PLoS Pathog 8:e1002584. https://doi.org/10.1371/journal.ppat.1002584

Pfeffer M (2015) Infektionsepidemiologie. In: Tiermedizinische Mikrobiologie, Infektions- und Seuchenlehre, vol 10. Enke, Stuttgart, pp 28–38

Pijlman GP et al (2008) A highly structured, nuclease-resistant, noncoding RNA produced by fla-viviruses is required for pathogenicity. Cell Host Microbe 4:579–591. https://doi.org/10.1016/j.chom.2008.10.007

Platonov A, Rossi G, Karan L, Mironov K, Busani L, Rezza G (2012) Does the *Japanese encephalitis virus* (JEV) represent a threat for human health in Europe? Detection of JEV RNA sequences in birds collected in Italy. Euro Surveill 17

Platt KB, Linthicum KJ, Myint KS, Innis BL, Lerdthusnee K, Vaughn DW (1997) Impact of *Dengue virus* infection on feeding behavior of *Aedes aegypti*. Am J Trop Med Hyg 57:119–125

Pompon J et al (2017) Dengue subgenomic flaviviral RNA disrupts immunity in mosquito salivary glands to increase virus transmission. PLoS Pathog 13:e1006535. https://doi.org/10.1371/journal.ppat.1006535

Proft J, Maier WA, Kampen H (1999) Identification of six sibling species of the *Anopheles maculipennis* complex (Diptera: Culicidae) by a polymerase chain reaction assay. J Parasitol Res 85:837–843

Puggioli A et al (2017) Does *Aedes albopictus* (Diptera: *Culicidae*) play any role in *Usutu virus* transmission in Northern Italy? Experimental oral infection and field evidences. Acta Trop 172:192–196. https://doi.org/10.1016/j.actatropica.2017.05.006

Raharimalala FN, Andrianinarivomanana TM, Rakotondrasoa A, Collard JM, Boyer S (2017) Usefulness and accuracy of MALDI-TOF mass spectrometry as a supplementary tool to identify mosquito vector species and to invest in development of international database. Med Vet Entomol 31:289–298. https://doi.org/10.1111/mve.12230

Rancès E, Johnson TK, Popovici J, Iturbe-Ormaetxe I, Zakir T, Warr CG, O'Neill SL (2013) The toll and Imd pathways are not required for *Wolbachia*-mediated *Dengue virus* interference. J Virol 87:11945–11949. https://doi.org/10.1128/JVI.01522-13

Ravanini P et al (2012) *Japanese encephalitis virus* RNA detected in *Culex pipiens* mosquitoes in Italy. Euro Surveill 17

Reisen WK, Hahn DC (2007) Comparison of immune responses of brown-headed cowbird and related blackbirds to *West Nile* and other *Mosquito-borne encephalitis viruses*. J Wildl Dis 43:439–449. https://doi.org/10.7589/0090-3558-43.3.439

Rothwell C et al (2009) Cholesterol biosynthesis modulation regulates dengue viral replication. Virology 389:8–19. https://doi.org/10.1016/j.virol.2009.03.025

Rudolf M (2015) Untersuchung der evolutionären Adaption von Orthobunyaviren an Insekten und Säugetierzellen. Dissertation, University of Bremen

Rudolf M et al (2013) First nationwide surveillance of *Culex pipiens* complex and *Culex torrentium* mosquitoes demonstrated the presence of *Culex pipiens* biotype *pipiens/molestus* hybrids in Germany. PLoS One 8:e71832. https://doi.org/10.1371/journal.pone.0071832

Saito K et al (2006) Specific association of Piwi with rasiRNAs derived from retrotransposon and heterochromatic regions in the Drosophila genome. Genes Dev 20:2214–2222. https://doi.org/10.1101/gad.1454806

Saiyasombat R, Bolling BG, Brault AC, Bartholomay LC, Blitvich BJ (2011) Evidence of efficient transovarial transmission of *Culex flavivirus* by *Culex pipiens* (Diptera: *Culicidae*). J Med Entomol 48:1031–1038

Sanchez-Vargas I et al (2009) *Dengue virus* type 2 infections of *Aedes aegypti* are modulated by the mosquito's RNA interference pathway. PLoS Pathog 5:e1000299. https://doi.org/10.1371/journal.ppat.1000299

Sanders HR, Foy BD, Evans AM, Ross LS, Beaty BJ, Olson KE, Gill SS (2005) *Sindbis virus* induces transport processes and alters expression of innate immunity pathway genes in the midgut of the disease vector, *Aedes aegypti*. Insect Biochem Mol Biol 35:1293–1307. https://doi.org/10.1016/j.ibmb.2005.07.006

Schaffner F, Mathis A (2014) Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis 14:1271–1280. https://doi.org/10.1016/S1473-3099(14)70834-5

Schaffner F, Kaufmann C, Pfluger V, Mathis A (2014) Rapid protein profiling facilitates surveillance of invasive mosquito species. Parasit Vectors 7:142. https://doi.org/10.1186/1756-3305-7-142

Schnettler E et al (2012) Noncoding flavivirus RNA displays RNA interference suppressor activity in insect and mammalian cells. J Virol 86:13486–13500. https://doi.org/10.1128/JVI.01104-12

Schnettler E et al (2013) Knockdown of piRNA pathway proteins results in enhanced *Semliki Forest virus* production in mosquito cells. J Gen Virol 94:1680–1689. https://doi.org/10.1099/vir.0.053850-0

Schulz C et al (2016) *Bluetongue virus* serotype 27: detection and characterization of two novel variants in Corsica, France. J Gen Virol 97:2073–2083. https://doi.org/10.1099/jgv.0.000557

Shchetinin AM, Lvov DK, Deriabin PG, Botikov AG, Gitelman AK, Kuhn JH, Alkhovsky SV (2015) Genetic and phylogenetic characterization of *Tataguine* and *Witwatersrand viruses* and other Orthobunyaviruses of the *Anopheles A*, *Capim, Guama, Koongol, Mapputta, Tete*, and *Turlock* serogroups. Viruses 7:5987–6008. https://doi.org/10.3390/v7112918

Sieg M et al (2017) Outbreak and cocirculation of three different *Usutu virus* strains in Eastern Germany. Vector Borne Zoonotic Dis 17:662–664. https://doi.org/10.1089/vbz.2016.2096

Sigfrid L et al (2017) Preparing clinicians for (re-)emerging arbovirus infectious diseases in Europe. Clin Microbiol Infect 24(3):229–239. https://doi.org/10.1016/j.cmi.2017.05.029

Sim S, Jupatanakul N, Ramirez JL, Kang S, Romero-Vivas CM, Mohammed H, Dimopoulos G (2013) Transcriptomic profiling of diverse *Aedes aegypti* strains reveals increased basal-level immune activation in *Dengue virus*-refractory populations and identifies novel virus-vector molecular interactions. PLoS Negl Trop Dis 7:e2295. https://doi.org/10.1371/journal.pntd.0002295

Sim S, Jupatanakul N, Dimopoulos G (2014) Mosquito immunity against arboviruses. Viruses 6:4479–4504. https://doi.org/10.3390/v6114479

Siomi MC, Sato K, Pezic D, Aravin AA (2011) PIWI-interacting small RNAs: the vanguard of genome defence. Nat Rev 12:246–258. https://doi.org/10.1038/nrm3089

Skalsky RL, Vanlandingham DL, Scholle F, Higgs S, Cullen BR (2010) Identification of microRNAs expressed in two mosquito vectors, *Aedes albopictus* and *Culex quinquefasciatus*. BMC Genomics 11:119. https://doi.org/10.1186/1471-2164-11-119

Slonchak A, Hussain M, Torres S, Asgari S, Khromykh AA (2014) Expression of mosquito microRNA Aae-miR-2940-5p is downregulated in response to *West Nile virus* infection to restrict viral replication. J Virol 88:8457–8467. https://doi.org/10.1128/JVI.00317-14

Smartt CT, Richards SL, Anderson SL, Erickson JS (2009) *West Nile virus* infection alters midgut gene expression in *Culex pipiens quinquefasciatus* Say (Diptera: *Culicidae*). Am J Trop Med Hyg 81:258–263

Smith JL, Fonseca DM (2004) Rapid assays for identification of members of the *Culex (Culex) pipiens* complex, their hybrids, and other sibling species (Diptera: *Culicidae*). Am J Trop Med Hyg 70:339–345

Souza-Neto JA, Sim S, Dimopoulos G (2009) An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense. Proc Natl Acad Sci U S A 106:17841–17846. https://doi.org/10.1073/pnas.0905006106

Sudeep AB, Bondre VP, Mavale MS, Ghodke YS, George RP, Aher RV, Gokhale MD (2013) Preliminary findings on *Bagaza virus* (Flavivirus: *Flaviviridae*) growth kinetics, transmission potential & transovarial transmission in three species of mosquitoes. Indian J Med Res 138:257–261

Talbalaghi A, Moutailler S, Vazeille M, Failloux AB (2010) Are *Aedes albopictus* or other mosquito species from northern Italy competent to sustain new arboviral outbreaks? Med Vet Entomol 24:83–87. https://doi.org/10.1111/j.1365-2915.2009.00853.x

Tappe D, Kapaun A, Emmerich P, Campos Rde M, Cadar D, Gunther S, Schmidt-Chanasit J (2014) *O'nyong-nyong virus* infection imported to Europe from Kenya by a traveler. Emerg Infect Dis 20:1766–1767. https://doi.org/10.3201/eid2010.140823

Terradas G, Joubert DA, McGraw EA (2017) The RNAi pathway plays a small part in *Wolbachia*-mediated blocking of *Dengue virus* in mosquito cells. Sci Rep 7:43847. https://doi.org/10.1038/srep43847

van den Hurk AF et al (2012) Impact of *Wolbachia* on infection with *Chikungunya* and *Yellow fever viruses* in the Mosquito Vector *Aedes aegypti*. PLoS Negl Trop Dis 6:e1892. https://doi.org/10.1371/journal.pntd.0001892

van Rij RP, Saleh MC, Berry B, Foo C, Houk A, Antoniewski C, Andino R (2006) The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. Genes Dev 20:2985–2995. https://doi.org/10.1101/gad.1482006

Velandia-Romero ML, Olano VA, Coronel-Ruiz C, Cabezas L, Calderón-Peláez MA, Castellanos JE, Matiz MI (2017) Detección del virus del dengue en larvas y pupas de *Aedes aegypti* recolectadas en áreas rurales del municipio de Anapoima, Cundinamarca, Colombia. Biomédica 37:193–200

Vodovar N, Bronkhorst AW, van Cleef KW, Miesen P, Blanc H, van Rij RP, Saleh MC (2012) Arbovirus-derived piRNAs exhibit a ping-pong signature in mosquito cells. PLoS One 7:e30861. https://doi.org/10.1371/journal.pone.0030861

Wagner S, Mathis A, Schonenberger AC, Becker S, Schmidt-Chanasit J, Silaghi C, Veronesi E (2018) Vector competence of field populations of the mosquito species *Aedes japonicus japonicus* and *Culex pipiens* from Switzerland for two *West Nile virus* strains. Med Vet Entomol 32(1):121–124. https://doi.org/10.1111/mve.12273

Waldock J, Olson KE, Christophides GK (2012) *Anopheles gambiae* antiviral immune response to systemic O'nyong-nyong infection. PLoS Negl Trop Dis 6:e1565. https://doi.org/10.1371/journal.pntd.0001565

Walker T et al (2011) The *wMel Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature 476:450–453. https://doi.org/10.1038/nature10355

Walther D, Kampen H (2017) The citizen science project 'Mueckenatlas' helps monitor the distribution and spread of invasive mosquito species in Germany. J Med Entomol 54:1790–1794. https://doi.org/10.1093/jme/tjx166

Wang XH et al (2006) RNA interference directs innate immunity against viruses in adult *Drosophila*. Science 312:452–454. https://doi.org/10.1126/science.1125694

Wang G et al (2012) Identifying the main mosquito species in China based on DNA barcoding. PLoS One 7:e47051. https://doi.org/10.1371/journal.pone.0047051

Waterhouse RM et al (2007) Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. Science 316:1738–1743. https://doi.org/10.1126/science.1139862

Weber F, Bridgen A, Fazakerley JK, Streitenfeld H, Kessler N, Randall RE, Elliott RM (2002) Bunyamwera bunyavirus nonstructural protein NSs counteracts the induction of alpha/beta interferon. J Virol 76:7949–7955

Weesendorp E, Loeffen W, Stegeman A, de Vos C (2011) Time-dependent infection probability of classical swine fever via excretions and secretions. Prev Vet Med 98:152–164. https://doi.org/10.1016/j.prevetmed.2010.11.010

Weitzel T, Gauch C, Becker N (2012) Identification of *Anopheles daciae* in Germany through ITS2 sequencing. J Parasitol Res 111:2431–2438. https://doi.org/10.1007/s00436-012-3102-8

Werner D, Kampen H (2013) The further spread of *Aedes japonicus japonicus* (Diptera, *Culicidae*) towards northern Germany. J Parasitol Res 112:3665–3668. https://doi.org/10.1007/s00436-013-3564-3

Werner D, Kronefeld M, Schaffner F, Kampen H (2012) Two invasive mosquito species, *Aedes albopictus* and *Aedes japonicus japonicus*, trapped in south-west Germany, July to August 2011. Euro Surveill 17

Wesson DM, Porter CH, Collins FH (1992) Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). Mol Phylogenet Evol 1:253–269

WHO (2017) http://www.who.int/mediacentre/factsheets/fs117/en/

Wilke AB, Christe Rde O, Multini LC, Vidal PO, Wilk-da-Silva R, de Carvalho GC, Marrelli MT (2016) Morphometric wing characters as a tool for mosquito identification. PLoS One 11:e0161643. https://doi.org/10.1371/journal.pone.0161643

Willard KA, Demakovsky L, Tesla B, Goodfellow FT, Stice SL, Murdock CC, Brindley MA (2017) *Zika virus* exhibits lineage-specific phenotypes in cell culture, in *Aedes aegypti* mosquitoes, and in an embryo model. Viruses 9. https://doi.org/10.3390/v9120383

Winter F, Edaye S, Huttenhofer A, Brunel C (2007) *Anopheles gambiae* miRNAs as actors of defence reaction against *Plasmodium* invasion. Nucleic Acids Res 35:6953–6962. https://doi.org/10.1093/nar/gkm686

Wittmann EJ, Baylis M (2000) Climate change: effects on *Culicoides*—transmitted viruses and implications for the UK. Vet J 160:107–117. https://doi.org/10.1053/tvjl.2000.0470

Xi Z, Ramirez JL, Dimopoulos G (2008) The *Aedes aegypti* toll pathway controls *Dengue virus* infection. PLoS Pathog 4:e1000098. https://doi.org/10.1371/journal.ppat.1000098

Xia H, Beck AS, Gargili A, Forrester N, Barrett ADT, Bente DA (2016) Transstadial transmission and long-term association of *Crimean-Congo hemorrhagic fever virus* in ticks shapes genome plasticity. Sci Rep 6:35819. https://doi.org/10.1038/Srep35819

Yan H, Zhou Y, Liu Y, Deng Y, Chen X (2014) miR-252 of the Asian tiger mosquito *Aedes albopictus* regulates *Dengue virus* replication by suppressing the expression of the *Dengue virus* envelope protein. J Med Virol 86:1428–1436

Yssouf A et al (2013a) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of tick vectors. J Clin Microbiol 51:522–528. https://doi.org/10.1128/JCM.02665-12

Yssouf A et al (2013b) Matrix-assisted laser desorption ionization—time of flight mass spectrometry: an emerging tool for the rapid identification of mosquito vectors. PLoS One 8:e72380. https://doi.org/10.1371/journal.pone.0072380

Yssouf A et al (2014) Identification of European mosquito species by MALDI-TOF MS. J Parasitol Res 113:2375–2378. https://doi.org/10.1007/s00436-014-3876-y

Yssouf A, Almeras L, Berenger JM, Laroche M, Raoult D, Parola P (2015) Identification of tick species and disseminate pathogen using hemolymph by MALDI-TOF MS. Ticks Tick Borne Dis 6:579–586. https://doi.org/10.1016/j.ttbdis.2015.04.013

Zambon RA, Nandakumar M, Vakharia VN, Wu LP (2005) The Toll pathway is important for an antiviral response in *Drosophila*. Proc Natl Acad Sci U S A 102:7257–7262. https://doi.org/10.1073/pnas.0409181102

Zambon RA, Vakharia VN, Wu LP (2006) RNAi is an antiviral immune response against a dsRNA virus in *Drosophila melanogaster*. Cell Microbiol 8:880–889. https://doi.org/10.1111/j.1462-5822.2006.00688.x

Zeller H (2012) Is Japanese encephalitis emerging in Europe? Euro Surveill 17

Zielke DE, Werner D, Schaffner F, Kampen H, Fonseca DM (2014) Unexpected patterns of admixture in German populations of *Aedes japonicus japonicus* (Diptera: *Culicidae*) underscore the importance of human intervention. PLoS One 9:e99093. https://doi.org/10.1371/journal.pone.0099093

Zielke DE, Ibanez-Justicia A, Kalan K, Merdic E, Kampen H, Werner D (2015) Recently discovered *Aedes japonicus japonicus* (Diptera: *Culicidae*) populations in The Netherlands and northern Germany resulted from a new introduction event and from a split from an existing population. Parasit Vectors 8:40. https://doi.org/10.1186/s13071-015-0648-1

Zielke DE, Walther D, Kampen H (2016) Newly discovered population of *Aedes japonicus japonicus* (Diptera: *Culicidae*) in Upper Bavaria, Germany, and Salzburg, Austria, is closely related to the Austrian/Slovenian bush mosquito population. Parasit Vectors 9:163. https://doi.org/10.1186/s13071-016-1447-z