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Chikungunya Virus: Role of Vectors in Emergence from Enzootic Cycles

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Abstract

Chikungunya virus (CHIKV), a re-emerging mosquito-borne arbovirus, has caused millions of cases of severe, often chronic arthralgia during recent outbreaks. In Africa, circulation in sylvatic, enzootic cycles involves several species of arboreal mosquito vectors that transmit among diverse nonhuman primates and possibly other amplifying hosts. Most disease occurs when CHIKV emerges into a human-amplified cycle involving *Aedes aegypti* and sometimes *Aedes albopictus* transmission and extensive spread via travelers. Epidemiologic studies suggest that the transition from enzootic to epidemic cycles begins when people are infected via spillover in forests. However, efficient human amplification likely only ensues far from enzootic habitats where peridomestic vector and human densities are adequate. Recent outbreaks have been enhanced by mutations that adapt CHIKV for more efficient infection of *Ae. albopictus*, allowing for geographic expansion. However, epistatic interactions, sometimes resulting from founder effects following point-source human introductions, have profound effects on transmission efficiency, making CHIKV emergence somewhat unpredictable.

1. INTRODUCTION

1.1. Chikungunya Virus

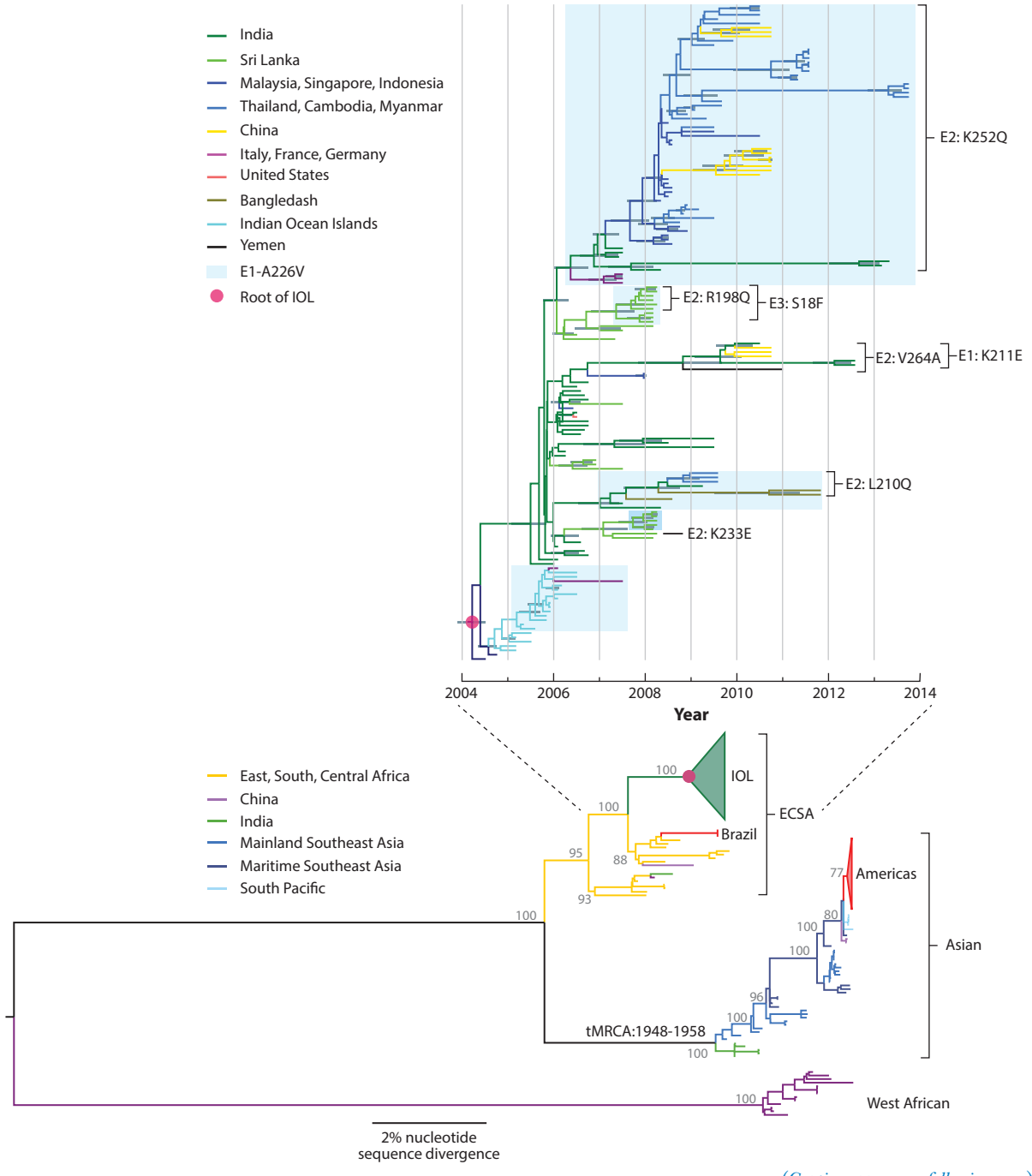
Chikungunya virus (CHIKV) is an arthropod-borne virus (arbovirus) transmitted by mosquitoes among vertebrates (138, 139) and a member of the family *Togaviridae*, genus *Alphavirus*. Although most CHIKV transmission is mosquito borne, vertical transmission from a pregnant woman to her infant during childbirth (perinatal) occurs regularly, often leading to severe central nervous system disease that can be fatal (41, 123); vertical transmission among mosquitoes has also been reported.

1.1.1. Disease. CHIKV causes a human disease syndrome, chikungunya fever (CHIKF), that typically includes fever, rash, and joint pains (arthralgia) that can be highly debilitating and chronic for years (139). Sequelae also include asthenia and mood changes, which further deteriorate the quality of life (93). Unlike many arboviral infections, including dengue and Zika, the majority of CHIKV infections are symptomatic, resulting in high attack rates during outbreaks. Although the case–fatality rate is typically <1%, mainly involving the elderly, it is underestimated in resource-poor countries with limited surveillance (86, 99). In addition to direct health impacts, medical management imposes a major economic burden along with loss of productivity and absenteeism (111).

1.1.2. Chikungunya virus evolution and emergence. CHIKV is believed to have evolved in Africa based on the close sister relationship with o'nyong-nyong virus, an alphavirus restricted to this continent. Phylogenetic analyses first identified three main lineages: West African enzootic, East/Central/South African (ECSA) enzootic, and Asian endemic/epidemic (91). The enzootic lineages, which are ancestral (**Figure 1**, **Table 1**), circulate in sub-Saharan forests, where non-human primates (NHPs) are amplification hosts, and several arboreal mosquitoes transmit (see Section 1.3). The ECSA lineage in particular is highly diverse, with deeply divergent lineages, some associated with epidemic transmission in Central Africa.

CHIKV has emerged from enzootic progenitors for centuries, transported on sailing ships in the past along with *Aedes* (*Stegomyia*) *aegypti* vectors to initiate human-amplified outbreaks in Asia and the Americas (47). However, phylogenetic analyses have only allowed the most recent emergences to be studied precisely. The Asian endemic/epidemic lineage, which was derived from an ECSA ancestor about a century ago (**Figure 1**), is associated with human-amplified transmission. This lineage was first isolated during the late 1950s and early 1960s and continues to circulate in Southeast Asia and Oceania today. Prior to 2005, the only mosquito definitively identified as an endemic/epidemic vector was *Ae. aegypti*, in South and Southeast Asia (**Figure 2**).

In 2004, another endemic/epidemic CHIKV lineage, first detected in coastal Kenya (24), emerged from an ECSA ancestor into the Indian Ocean basin to cause epidemics on several islands. Later, the same Kenyan strain, the ancestor of this new Indian Ocean lineage (IOL), spread independently into South Asia, followed by Southeast Asia (135). The IOL continues to circulate in Asia more than 10 years after its arrival. During both the Indian Ocean and South Asian outbreaks, thousands of infected travelers transported CHIKV nearly throughout the world, including to Europe to initiate small outbreaks in France and Italy (98), the first detected in temperate climates during the past century, as well as to the Americas, including dengue-endemic locations highly permissive for CHIKV due to the abundant *Ae. aegypti* populations and completely naïve human populations. However, autochthonous CHIKF was not detected in the Americas until 2013, when an Asian Lineage strain was detected in the Caribbean Island of St. Martin (20). This outbreak spread quickly into Central, South, and North America, where major epidemics peaked in 2016



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Phylogenetic trees of chikungunya virus (CHIKV) derived using Bayesian analyses and genomic viral sequences. The lower tree shows relationships of major lineages, and the upper tree depicts more detailed relationships within the Indian Ocean lineage (IOL), as well as the locations and timing of mosquito vector-adaptive mutations listed in **Table 1**. These include E1-A226V, which is present in light blue shaded clades and evolved convergently at least four times, consistent with its strong selective advantage for transmission by *Aedes albopictus*. Other *Ae. albopictus*–adaptive mutations, as well as epistatic residues in the E1, E2 and E3 envelope glycoproteins (*black labels*), apparently only evolved once. Figure adapted with permission from Reference 21. Abbreviations: CHIKV, chikungunya virus; ECSA, East/Central/South African; IOL, Indian Ocean lineage.

but where circulation continues in many locations. Since 2013, CHIKV has continued to spread from Africa to initiate outbreaks in southern France and Brazil, and resurging IOL strains have spread from recent epidemics in India and/or Pakistan to initiate renewed transmission in Italy and France (99, 134). Due to limited surveillance, the total burden of CHIKF is unknown, but estimates of tens of millions of cases since 2005 are realistic.

1.2. Mosquito Biology and Transmission

The ability of arthropods to transmit pathogens depends on intrinsic and extrinsic factors and is expressed in two terms: (*a*) Vector competence, the ability of a vector to become infected and transmit after the pathogen is ingested in a blood meal, is often regulated for arboviruses at the level of midgut infection (60), and (*b*) vectorial capacity, the number of infective bites arising from

Table 1 Amino acid substitutions in IOL CHIKV shown to affect transmission by urban mosquito vectors

Year first detected	Protein	Substitution	CHIKV genotype	Fitness effect on *Aedes albopictus* transmission	Fitness effect on *Aedes aegypti* transmission	Reference(s)
2005	E1	A226V	IOL	40-fold increase	Slight decrease	108, 129, 132
			Asian	None detected	None detected	124
2007	E2	K252Q	IOL	Eightfold increase	None detected	129
			Asian	None detected	None detected	129
2008	E2	K233E	IOL	Sixfold increase	None detected	126
			Asian	None detected	None detected	126
2008	E2/E3	R198Q/S18F	IOL	16-fold increase	None detected	126
			Asian	None detected	None detected	126
2009	E2	L210Q	IOL	Fivefold increase	None detected	126
			Asian	None detected	None detected	126
2010	E1/E2	K211E/V264A	IOL	None detected	Slight increase	2
1958	E1	A98T[1]	IOL	None detected (epistatic, threonine prevents penetrance of E1-A226V)	None detected	124
Unknown	E2	I211T[2]	ECSA	None detected (epistatic, threonine allows penetrance of E1-A226V)	None detected	127

[1]Threonine is found only in Asian lineage strains and is believed to be the result of a founder effect when an ancestral ECSA strain was introduced somewhere in Asia.

[2]E2-211 appears to be polymorphic among ECSA strains. The IOL lineage originated from an ECSA strain with threonine, while the Brazil strain introduced in 2013 has an isoleucine.

Abbreviations: CHIKV, chikungunya virus; ECSA, East/Central/South African lineage; IOL, Indian Ocean lineage.

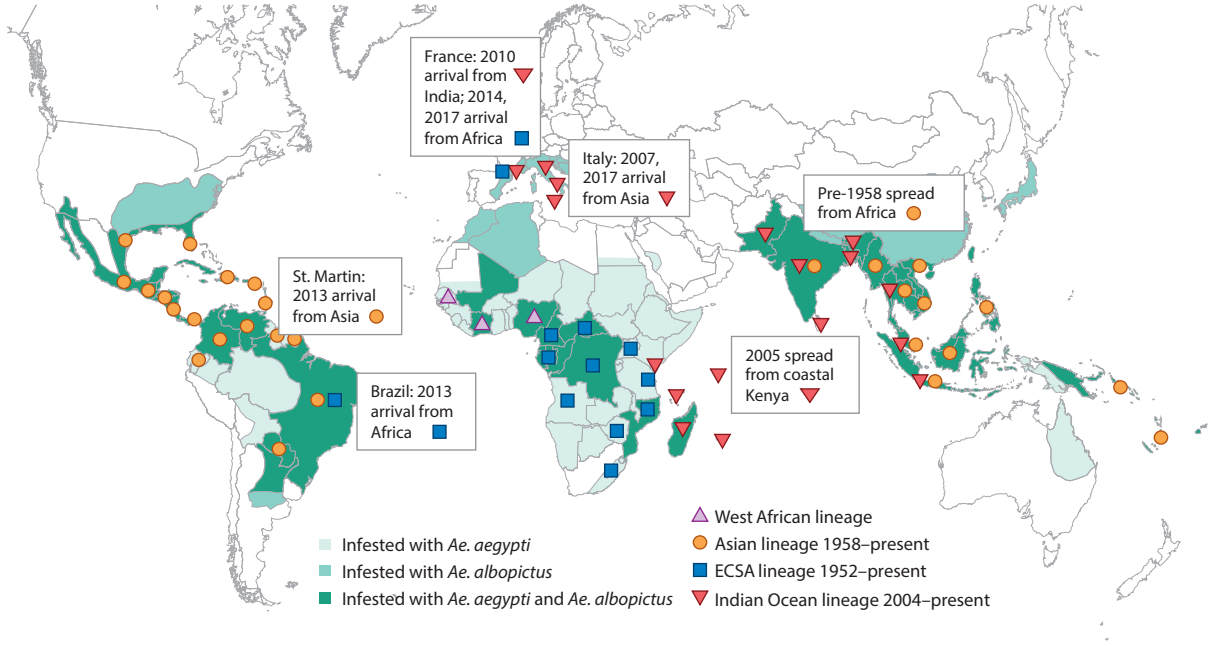


Figure 2

Map showing the temporal and geographic distribution of major CHIKV lineages. Figure adapted with permission from Reference 99. Abbreviations: *Ae.*, *Aedes*; CHIKV, chikungunya virus; ECSA, East/Central/South African.

an infected host, a more comprehensive measurement of the efficiency of transmission defined as

$$VC = \frac{ma^2bp^n}{-log_e p},$$

where m is the number of female mosquitoes per host, a is the daily blood feeding rate, b is the transmission rate among exposed vectors, p is the probability of daily vector survival, and n is the extrinsic incubation period (time between virus ingestion and transmission competence) (65).

Host-seeking female mosquitoes are infected after feeding on a viremic animal. CHIKV first replicates in the midgut, then enters the hemocoel before disseminating to the salivary glands. Midgut basal lamina reorganization during blood digestion mediates this dissemination process (38). The extrinsic incubation period is generally 2–5 days (80), suggesting that even vector populations with poor daily adult survival can transmit effectively. Females with disseminated virus in their salivary glands can transmit by injecting infectious saliva into a naïve host during a subsequent blood meal, leading to horizontal transmission. *Ae. aegypti* feeding is often interrupted when it is disturbed during blood feeding, and it may then complete the meal on one or more hosts in the vicinity. This can lead this highly anthropophilic species to feed on multiple persons daily, increasing the risk of CHIKV infection and transmission to multiple hosts, greatly enhancing vectorial capacity (89).

Vector competence of *Ae. aegypti* and *Aedes albopictus* shows variation according to the geographical origin of the mosquito population (10, 66, 119, 130) and CHIKV strain (10, 66, 79, 119, 130). Some studies show that *Ae. albopictus* is more competent than *Ae. aegypti* (119, 130). Oral CHIKV exposure to several other mosquito species (*Aedes furcifer*, *Aedes fulgens*, *Aedes vittatus*, *Mansonia africana*, *Aedes calceatus*, *Culex horridus*, *Eretmapodites chrysogaster*, *Aedes metallicus*, *Aedes*

ledgeri, *Aedes circumluteolus*, and *Aedes simpsoni*) revealed susceptibility only for *Ae. vittatus* and *Ae. furcifer* (25, 31, 32).

CHIKV has also been shown experimentally to disseminate to the mosquito ovaries, leading to infected offspring eggs (1, 23, 142). This transovarial transmission, a form of vertical transmission, may allow for viral maintenance during adverse conditions when the vector cannot continue its developmental cycle. This mechanism also allows the vector to play the role of reservoir during interepidemic periods. Natural vertical CHIKV transmission has been detected in adult *Ae. albopictus* emerged from larvae (28, 52, 81, 95) and from field-collected male *Ae. aegypti*, *Ae. albopictus* (39, 120), and *Ae. furcifer* (34, 36). Moreover, infected *Ae. aegypti* males are able to infect females during mating (venereal transmission) (70).

After blood digestion and egg maturation, gravid female mosquitoes seek suitable oviposition sites. Usually, *Ae. aegypti* does not disperse more than 800 m (63, 96), so it does not play an important role in CHIKV dissemination. *Ae. aegypti* mainly uses discarded containers located inside or around human habitations for oviposition. Adult females are highly endophilic, endophagic, and anthropophilic, further contributing to high vectorial capacity. *Ae. albopictus* uses a mixture of natural and artificial larval habitats and thus can coexist with *Ae. aegypti* (9, 109).

1.3. Enzootic Transmission Cycles

CHIKV circulates in forested regions of sub-Saharan Africa in an ancestral transmission cycle, known as enzootic or sylvatic, where it is transmitted by arboreal, canopy-dwelling *Aedes* spp. vectors among NHPs. This cycle was first identified based on CHIKV isolation from wild *Aedes africanus* mosquitoes in Uganda in 1958, where experimental studies showed monkey viremia and seroconversion postinoculation (73, 83, 141), and CHIKV or anti-CHIKV antibody was detected in many animals, mainly NHPs. Enzootic CHIKV exhibits regular, periodic amplification detected by mosquito surveillance, punctuated by interepizootic silence (**Figure 3**). In Senegal, amplifications occur every 3–5 years (36), and humans are considered incidental hosts infected in forests by sylvatic vectors or by vectors (*Ae. furcifer* in West and South Africa, *Ae. africanus* in East and Central Africa) invading villages (34, 36, 55). However, only sporadic human cases and small rural outbreaks are observed in enzootic regions (92).

In Africa, CHIKV has been isolated from many NHPs, including bushbabies (*Galago senegalensis*) and monkeys (*Cercopithecus aethiops*, *Chlorocebus sabaeus*, *Erythrocebus patas*, *Papio papio*) (36, 54, 78). In addition, seroprevalence studies reported antibodies in diverse NHP species including chimpanzees (*Pan troglodytes*), colobus monkeys (*Colobus a. abyssinicus*), baboons (*Papio dogueri*, *Papio ursinus*, *Papio anubis*, *Papio cynocephalus*, *P. papio*), mangabeys (*Cercocebus* sp.), vervets (*C. aethiops*, *Cercopithecus mitis*, also known as African green monkeys), African red monkeys (*E. patas*), mandrills (*Mandrillus sphinx*), and red-tailed monkeys (*Cercopithecus ascanius*) (4, 56, 68) in the Belgian Congo, Ethiopia, Uganda, South Africa, Zimbabwe, Central African Republic, Senegal, Kenya, and Gabon. The susceptibility of *C. aethiops pygerythrus* and *P. ursius* was demonstrated experimentally (73, 74).

In Asia, the existence of enzootic CHIKV remains unproven, although NHPs and a wide range of seropositive vertebrates have been found in Thailand, Malaysia, and the Philippines (48, 51, 67). Supporting the potential existence of an Asian enzootic cycle, four CHIKV strains from *Macaca fascicularis* in Malaysia were isolated in 2008–2009 (5). However, these strains could represent spillback from ongoing human-amplified transmission, since serosurveys did not indicate widespread NHP infection (106). Further, seroprevalence in Sri Lankan *Macaca sinica* was negative (84). These data, combined with the fact that the virus in Asia has been isolated only from domestic vectors (48), suggest no Asian enzootic cycle, and that only endemic, human-amplified CHIKV occurs there.

Country	Species
Senegal	*Ae. africanus; Ae. centropunctatus; Ae. dalzieli; Ae. hirsutus; Ae. iritans; Ae. luteocephalus; Ae. metallicus; Ae. neoafricanus; Ae. furcifer; Ae. taylori; Ae. argenteopuctatus; Ae. vittatus; An. coustani; An. domicola; An. funestus; An. gambiae; An. rufipes; Cx. ethiopicus; Cx. poicilipes; Ma. uniformis*
Cote d'Ivoire	*Ae. africanus; Ae. luteocephalus; Ae. furcifer; Ae. argenteopunctus; Ae. vittatus; Ae. abnormalis; Ae. cumminsii; Ae. gr. mutilus; Ae. cordellieri; Ae. ingrami; Ae. grahami; Ae. jamoti; Ae. gr. palpalis; Ae. taeniorostris; Ae. opok; Ae. usambara; An. funestus; An. nili; Ma. uniformis; Ma. africana; Er. gr. inornatus; Cx. gr. decens; Cx. guiarti; Cx. quinquefasciatus; Cx. weschei; Cx. cinereus; Cq. maculipennis*
Burkina Faso	*Ae. africanus; Ae. luteocephalus; Ae. furcifer*
CAR	*Ae. africanus; Ae. vittatus; Ae. opok; An. funestus; Ma. africana*
Uganda	*Ae. africanus; Ma. africana; Cq. fuscopennata*
Tanzania	*Ae. africanus ; Ae. aegypti*
South Africa	*Ae. furcifer*
Nigeria	*Ma. africana*
Mali	*Ae. furcifer*
Gabon	*Ae. aegypti; Ae. albopictus*
Rep. Congo	*Ae. aegypti; Ae. albopictus*
Angola	*Ae. aegypti*
La Reunion	*Ae. albopictus; Cx. quinquefasciatus*
Mauritius	*Ae. albopictus*
Comoros	*Ae. aegypti*
Madagascar	*Ae. albopictus*

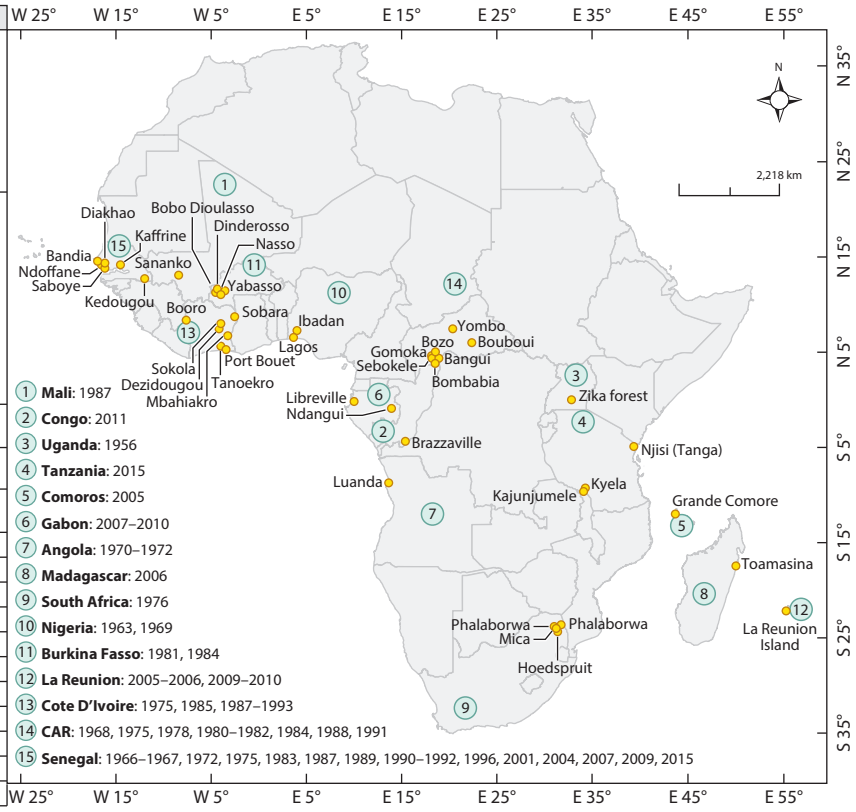


Figure 3

CHIKV isolations from sylvatic and domestic mosquitoes in Africa. Yellow circles represent the exact locations where CHIKV was detected, and green circled numbers indicate countries and years of CHIKV amplification and detection. Figure adapted with permission from Reference 32. Abbreviations: *Ae.*, *Aedes*; *An.*, *Anopheles*; CAR, Central African Republic; CHIKV, chikungunya virus; *Cq.*, *Coquillettidia*; *Cx.*, *Culex*; *Er.*, *Eretmapodites*; *Ma.*, *Mansonia*.

Beyond NHPs, CHIKV has been isolated in Africa from bats (18), rodents, palm squirrels, and birds (13, 36, 59), and antibodies were detected in rats (136), birds, reptiles, and elephants (56). Experimental infections demonstrated the development of viremia in bats (12, 55), rodents, and reptiles (11, 73). These fauna may therefore contribute to secondary cycles, which could explain CHIKV persistence in enzootic regions where NHPs undergo slow population turnover, and therefore high herd immunity after amplifications, and a short duration of viremia (118). In addition, vertical transmission in mosquitoes is unlikely to be efficient enough for long-term CHIKV maintenance.

1.4. Endemic/Epidemic Transmission Cycles

Endemic/epidemic CHIKV transmission has occurred at least for centuries in Africa, Asia, and the Americas (19, 47), probably since both the virus and *Ae. aegypti* were transported from Africa on sailing ships thanks in part to their desiccation-resistant eggs (15). The storage of water, providing suitable larval habitats, combined with susceptible crews and sometimes slaves, provided all necessary components for onboard transmission. Upon arrival in port cities either already inhabited by *Ae. aegypti* or susceptible to transient colonization, e.g., temperate cities, transmission could move ashore, sparking outbreaks.

2. ECOLOGY AND BEHAVIOR OF VECTORS

2.1. Enzootic Vectors

In Africa, CHIKV has been detected in 41 mosquito species (**Figure 3**) belonging to the genera *Aedes* (23 species), *Anopheles* (6), *Coquillettidia* (2), *Culex* (7), *Eretmapodites* (1), and *Mansonia* (2). Although only a few of these have been demonstrated to be competent vectors, mosquitoes found infected with CHIKV in the field can be classified into three groups according to the number of localities where they were found infected and the frequency of infection. The first group, considered major vectors, includes *Ae. aegypti*, *Ae. africanus*, *Ae. furcifer*, *Aedes luteocephalus*, *Aedes opok*, *Ae. vittatus*, *Aedes argenteopunctatus*, *Anopheles funestus*, *Ma. africana*, and *Mansonia uniformis*. The second group, considered potential secondary vectors that are associated with CHIKV for several years, but only in one locality, includes *Aedes taylori*, *Aedes dalzieli*, *Aedes grahami*, *Aedes neoafricanus*, *Anopheles gambiae*, *Culex quinquefasciatus*, and *Eretmapodites inornatus*. Members of the third group are minor or accessory vectors found infected only one time in one country.

The species composition of the main CHIKV vectors varies geographically (32) (**Figure 3**). Most enzootic CHIKV vectors (*Ae. aegypti*, *Ae. africanus*, *Ae. furcifer*, *Ae. luteocephalus*, *Ae. taylori*, and *Ae. opok*) oviposit in tree holes and fruit husks, while others (*Ae. vittatus*, *Ae. argenteopunctatus*, *An. funestus*, *Ma. africana*, and *Ma. uniformis*) use rock holes, ponds, pools, and small streams in forest galleries and savanna as larval habitats (33). Most potential CHIKV vectors are primatophilic (NHPs and humans), crepuscular, and outdoor feeders (34), while others like *Ae. vittatus* and *Ae. argenteopunctatus* are more generally mammalophilic. *Ae. furcifer*, *Ae. vittatus*, *Ae. africanus*, and *Ae. dalzieli* feed readily on humans within villages near sylvatic habitats (30, 35, 72).

2.2. Endemic/Epidemic Vectors

Ae. aegypti and Ae. albopictus are the only mosquitoes believed to regularly transmit CHIKV in settings involving human amplification. *Ae. aegypti* evolved in African forests and is today present throughout much of the tropics and subtropics. In Africa, it occurs in two distinct forms: (*a*) the darker, ancestral, forest-dwelling *Ae. Ae. formosus*, which uses natural habitats (rock holes, tree holes, fruit husks, etc.) for oviposition and larval development and has a zoophilic tendency, and (*b*) the domesticated *Ae. Ae. aegypti*, which is widespread beyond Africa, highly anthropophilic, and found mainly in urban environments, where it uses artificial containers for oviposition (40, 71, 117). Thanks to the behavioral and ecological traits described above, *Ae. Ae. aegypti* is the principal endemic/epidemic vector of yellow fever (76), dengue (45), Zika virus (46) and CHIKV (37).

Early studies showed that *Ae. Ae. aegypti* evolved from *Ae. Ae. formosus* and also exists in Africa but with a distribution limited to the eastern coast (90, 117). The presence of this domesticated form in West and Central Africa is still questionable due to the lack of reliable identification methods, although recent studies suggested that both forms exist in West Africa (116). Differentiation based on overall color is subjective, while the presence of scales on the first abdominal tergite is considered more informative. Genetic studies indicate multiple historic African domestication events, with *Ae. Ae. formosus* exhibiting considerable ecologic variability and occurring nearly sympatrically with the domesticated form in some locations (16).

Ae. albopictus, a close relative of *Ae. aegypti* in the subgenus *Stegomyia*, originated in the forests of Southeast Asia (110) but has recently invaded peri-urban, rural, and forested areas on five continents, probably through the transport of eggs in used tires and other artificial containers that it exploits for larval development. It can colonize both temperate regions, thanks to its diapausing, cold-resistant eggs (50), and tropical regions (64) and is now abundant in the Americas, Europe, and Africa (58).

Prior to 2005, *Ae. ae. aegypti* was recognized as the only important vector for urban CHIKV transmission, including in the first recognized African outbreak (103), as well as early outbreaks in South (94) and Southeast Asia (49). Only one early study in the Calcutta region provided circumstantial evidence for *Ae. albopictus* transmission (42). This species also serves as a secondary vector for dengue (61) and Zika viruses (7, 44) in regions where *Ae. aegypti* cannot survive winters and other locations where it does not thrive. Its secondary role as a CHIKV vector of most strains is probably explained by its less anthropophilic behavior, fewer partial blood meals, and other characteristics that limit vectorial capacity compared to *Ae. aegypti*. Since 2005, *Ae. aegypti* continues to serve as the primary endemic/epidemic vector in most locations. However, in temperate regions where *Ae. aegypti* does not occur, as well as some tropical, less urban locations, *Ae. albopictus* is more abundant and can serve as the primary CHIKV vector (25).

In La Reunion in 2005, *Ae. albopictus* was incriminated as the principal CHIKV vector during an outbreak (132). There, *Ae. aegypti* has decreased in abundance during recent decades and is not found in artificial containers, as is typical for domestic forms of this species in most locations (9). During the outbreak, viral sequencing identified the gradual replacement of alanine at E1 envelope protein residue 226 by valine (108). Because this residue can affect alphavirus infection of mosquito cells, this substitution was hypothesized to affect mosquito transmission. The E1-A226V substitution was subsequently shown to have a dramatic impact on increasing infection of *Ae. albopictus*, with little effect on infection of *Ae. aegypti* (128, 132). Subsequent studies showed that E1-A226V occurred convergently in at least some other locations as the IOL strain spread to regions of Asia with large populations of *Ae. albopictus* (**Figure 1**), supporting its selective advantage (27). In contrast, IOL strains in locations dominated by *Ae. aegypti* retained the ancestral E1-226A, as have some strains involved in short-term *Ae. albopictus*–borne outbreaks (2).

The adaptive evolution of CHIKV did not end with E1-226. E2 protein mutations detected during spread through South Asia from 2007 to 2008 (**Figure 1**, **Table 1**) were also tested for their effect on urban vector infection, and three of these, like E1-A226V, were found to confer further fitness gains for *Ae. albopictus* but not *Ae. aegypti* (126, 129). Furthermore, the common pattern of substitutions of all of these amino acids by glutamine or glutamic acid, within the same acid-sensitive E2 region that is involved in a major conformational change within E1/E2 dimers required for endosomal fusion for viral entry, allowed additional artificial substitutions of other amino acids in the same region to be tested to determine if the pattern was predictive. Several of these artificial substitutions, never detected in natural CHIKV strains, also conferred fitness gains in *Ae. albopictus* but not *Ae. aegypti.* Furthermore, the experimental combination of two of the natural E2 mutations, not seen together in any natural CHIKV strain, resulted in further fitness gains for *Ae. albopictus*, also indicating the potential for further CHIKV adaptation to this invasive vector (126). Finally, in 2010, a combination of E1-K211E and E2-V264A substitutions detected in India was shown to slightly increase IOL CHIKV titers in infected *Ae. aegypti* (2).

A major enigma related to the selective advantage of E1-226V for *Ae. albopictus* was that, despite the Asian CHIKV lineage circulating in its native territory for many decades, this residue was not found in any Asian lineage strains (**Figure 1**). Reverse genetic studies determined that a single epistatic residue, E1-98, strongly influences the E1-226–mediated phenotype; E1-98A, found in all African and IOL strains, permits the *Ae. albopictus*–adaptive phenotype, while E1-98T, found in all Asian lineage strains but in no other lineages, prevents its penetrance (**Table 1**). The lack of an E1-98–mediated phenotype of their own for vectors and mice suggests that the threonine residue was introduced into Asia as a founder effect. Founder effects are probably common during CHIKV spread because point source introductions, indicated by phylogenetic studies, probably typically involve a single infected traveler, and both infection of the mosquito midgut and transmission via saliva include strong viral population bottlenecks (125). Additional evidence of the major impacts

of founder effects on CHIKV emergence come from studies of the 3′ untranslated genome region. Evolutionary reconstructions accompanied by reverse genetic studies indicate that the Asian lineage progenitor arrived from Africa with a debilitating deletion, which was gradually but only partially restored to its ancestral fitness through a series of duplications and point mutations (22). An additional duplication that also improved fitness for mosquito cell replication occurred upon introduction of an Asian strain to the Americas in 2013 (114).

In addition to amino acid E1-98, another epistatic residue that influences the penetrance of E1-A226V was identified in reverse genetic studies. Residue 211 of the E2 protein is either isoleucine or threonine in natural CHIKV strains (**Table 1**). ECSA strains are polymorphic for these two residues; reverse genetic studies demonstrate that strains with threonine undergo a major E1-A226V–mediated fitness increase for infection of *Ae. albopictus*, while strains with E2-211I do not respond to this substitution (127). Thus, the IOL with E2-211T readily underwent adaptive E1-A226V–based evolution. Fortunately, the ECSA strain introduced recently into Brazil had E2-211I and is thus predicted to not readily adapt for transmission by this vector (137). Thus, both Asian/American and ECSA/Brazilian strains circulating in the Americas are less likely to adapt for transmission by *Ae. albopictus* than IOL strains, which have not yet been detected circulating in the region; the lack of E1-226V in any New World CHIKV sequences supports these predictions. Another constraint on the A226V-mediated adaptation is the geographic source of *Ae. albopictus*; mosquitoes from the Democratic Republic of Congo are affected by the E1-226V mutation (133).

3. MECHANISMS OF EMERGENCE

3.1. Exposure of People to Enzootic Transmission Cycles

The interconnection between enzootic CHIKV cycles and the initiation of epidemic transmission remains obscure but could be established by humans or enzootic hosts introducing the virus into nearby villages after infection in the forest or through dispersal of forest mosquitoes. In either case, the presence of a vector with domestic activity would be essential for human-amplified transmission to ensue. If peridomestic transmission occurs, equal exposure to CHIKV, regardless of gender, age, or social status, would be expected, unlike in sylvatic exposure, where vectors likely have differential contact with certain population groups that frequent forests.

To assess the risk of human exposure to enzootic CHIKV spillover and identify high-risk environments (including barren land, villages, forest, savanna, and agricultural lands), geospatial analyses of anthropophilic mosquitoes were undertaken in Kedougou, Senegal (12°11′00 W; 12°33′00 N) (34). The relationship between vector density and land cover classes and the impact of proximity to forests on human exposure were estimated. Two groups of vectors were delineated; one includes *Ae. vittatus*, *Ae. dalzieli*, *Ae. furcifer*, and *Ae. Ae. formosus*, which have high ecological plasticity (**Figure 4**). While living in the forest, these species are also active in nearby villages. The second includes *Ae. africanus*, *Ae. luteocephalus*, and *Ae. taylori*, which are mainly confined to forests, with an apparent preference for the canopy. However, CHIKV was detected in mosquitoes collected in all land cover classes, including seven forests, three savanna regions, three barren regions, two agricultural regions, and three villages (**Figure 5**). There was a significant correlation between total vector abundance and the number of CHIKV-positive pools across sites. These findings revealed potential human exposure among all land cover classes (34).

Although some infected enzootic vectors frequent human dwellings, peridomestic human infections are poorly supported by the available data. Evidence in favor of direct enzootic spillover in forests includes the sporadic pattern of human cases over time and space, higher seroprevalence in NHPs than in humans, and differences in human seroprevalence according to age and occupational activity in forests.

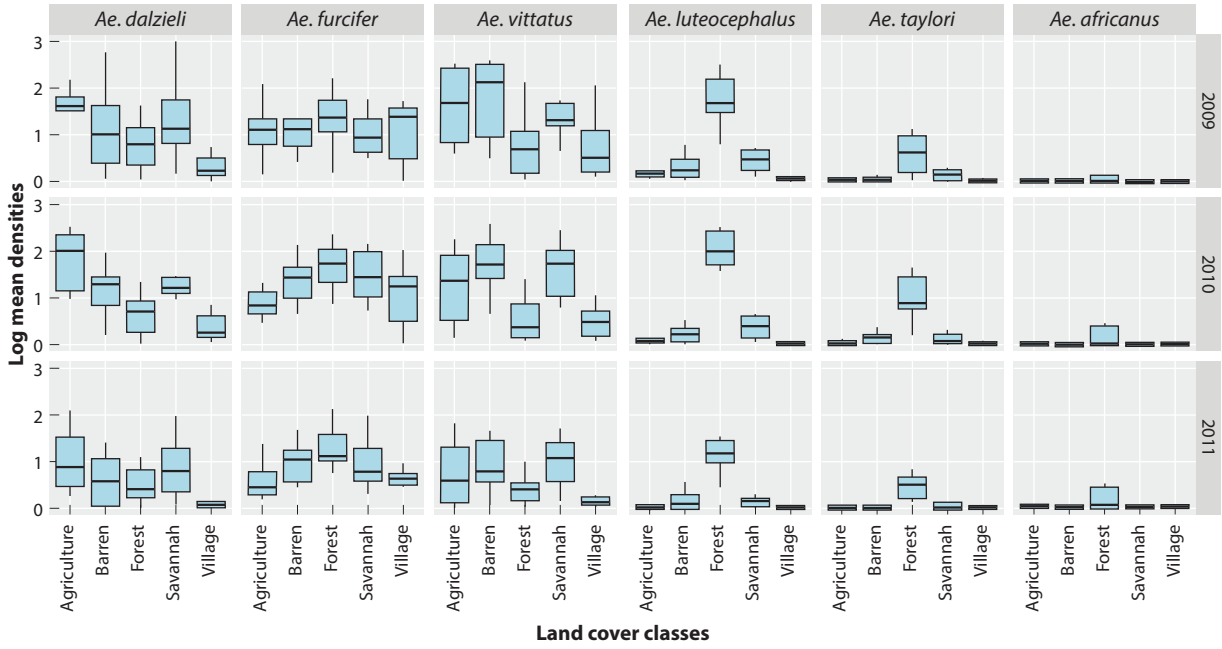


Figure 4

Densities of chikungunya virus vectors in the different landscape classes in Kedougou, southeastern Senegal. Abbreviation: *Ae.*, *Aedes*. *Y*-axis density units are the number of female mosquitoes landing per person per evening.

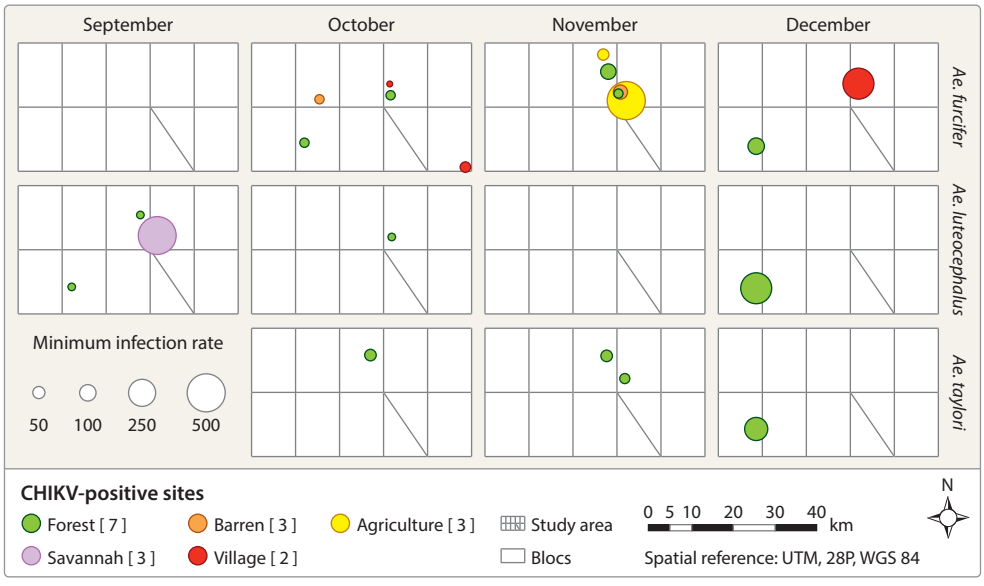


Figure 5

Spatiotemporal dynamics of CHIKV minimum infection rates at Kedougou, southeastern Senegal, in 2009. The estimated number of CHIKV-positive mosquitos per 1,000 tested was estimated using the pooled infection rate program (PooledInfRate, version 3.0, Centers for Disease Control and Prevention, Fort Collins, CO; **https://www.cdc.gov/westnile/index.html**) with a scale of 1,000. Abbreviations: *Ae.*, *Aedes*; CHIKV, chikungunya virus.

In Senegal, while a stable enzootic cycle exists in the southeast, most human outbreaks have been detected in western regions (14, 36, 88, 101, 121). In enzootic Kedougou, human infection is reported based on opportunistic surveillance (77, 104, 113, 121), and the number of infections remains poorly characterized. A 2009 epizootic study showed low CHIKF incidence (0.55–9.38/1,000) (113) compared with that reported in Kaffrine (35.3%) in western Senegal in 1996–1997 (121). Potential explanations for this difference include the lower human density in Kedougou (8 people/km^2) than the estimated 3,000–7,000/km^2 required for sustained transmission of dengue virus, which has an identical urban cycle (107). An absence of CHIKF cases was detected among children under 4 years of age, who generally remain in the household, compared to adults and older children, who are more frequently exposed to forests for agriculture and gold extraction (113). A limitation of the Kedougou surveillance is that asymptomatic and mild cases that did not seek health care were not sampled. Concurrent serosurveys of NHPs revealed CHIKV-neutralizing antibodies in 87% of Guinea baboons, 75% of African green monkeys (*Ch. sabaeus*), and 71% of patas monkeys (*E. patas*), including 34% of infants and juveniles (4).

In other African countries where enzootic CHIKV is documented, diverse epidemiological profiles are reported. No outbreak was reported in Burkina Faso following CHIKV detection in mosquitoes in 1981 and 1984 at Dinderosso and Yabosso, respectively (26, 100). In the Central African Republic, CHIKV was isolated from humans in 1978, 1982–1984, 1987, and 1995 (69, 105) in Bozo and Bangui. Human seroprevalence averaged 17% in 1978–1979 in several parts of the country. In Cote d'Ivoire, anti-CHIKV immunoglobulin (Ig) M and IgG were detected from a 1997 traveler (87). A 1998 serosurvey in 21 villages of western Côte d'Ivoire showed 9.9% CHIKV positivity (6). Five cases of CHIKF were detected in the Entebbe area during a 1968 epizootic (72), and another outbreak occurred in the Kampala region of Uganda in 1982 (57). A 1984 serosurvey showed that CHIKV (47%) was the most prevalent arbovirus in villages of the Karamojat district (102). In Nigeria, CHIKF outbreaks were reported in Ibadan in 1969 and 1974 (78, 122). In 2008, neutralizing antibodies against CHIKV were detected in 17.4% of febrile patients tested in Borno State (8) while in Maiduguri, IgM and IgG were detected in 10.5% of sera (78), with 6.5% positive for IgM only and the remainder positive for both IgM and IgG (3).

CHIKV has also caused outbreaks in many countries in East Africa (Uganda, Mozambique, Kenya, and Tanzania), the Indian Ocean basin (Reunion Island, Seychelles, Mauritius, and Madagascar), southern Africa (Zimbabwe and Angola), Central Africa (the Central African Republic, Democratic Republic of Congo, Cameroon, and Gabon) and West Africa (Senegal, Nigeria, and Guinea) (53, 62, 82, 85, 86, 115). However, these outbreaks occurred in urban environments with *Ae. Ae. aegypti* and/or *Ae. albopictus*, the latter of which is rapidly extending its distribution in Africa, as the primary vectors.

3.2. Spread Following Enzootic Spillover

There is good evidence from the medical literature that CHIKV regularly spread from Africa to islands off the eastern coast, as well as to Asia and the Americas, at least as far back as the eighteenth century (19, 47). Asian lineage strains became widespread, but strain relationships suggested limited spread, for example, between India and Southeast Asia. However, detailed phylogenetic analyses of Asian lineage strains also suggest frequent movement between mainland Southeast Asia and nearby islands such as the Philippines and the South Pacific (21).

After its emergence in a remote enzootic region, CHIKV probably typically remains localized without further spread because the conditions facilitating human amplification are not present (insufficient susceptible populations or peridomestic vectors). Spread to more urban regions could occur with vector dispersal, but it is more likely that human travel is involved in spread (75).

Phylogenetic analyses delineating CHIKV spread since 2004 suggest introductions via infected persons, mostly representing point sources. For example, in the Indian Ocean, 2005–2006 outbreaks in Comoros, La Reunion, and the Seychelles were probably initiated by individual infected persons from Kenya in 2004, as was spread of the same strain from Kenya to South Asia (135). Likewise, the Asian and ECSA strains were also likely introduced into the Caribbean (20) and Brazil (112), respectively, in 2013 by individual infected travelers, followed by extensive amplification and spread due to the abundance of *Ae. aegypti* and naïve human populations. Fortunately, as described in Section 2.2, both American founder strains had epistatic constraints on the adaptation to *Ae. albopictus*; the initial predictions of lack of adaptive potential have been supported by the lack of any known adaptive mutations in American strains, as well as the lack of evidence for transmission by this species, including in temperate regions. Smaller European outbreaks in Italy and France were also attributed to traveler introductions from South Asia and Africa (17, 29, 43, 98, 131). These outbreaks involved transmission of strains, both with and without adaptive mutations, by *Ae. albopictus* but were probably limited by both the seasonality of transmission-permissive temperatures and lower levels of human contact with the vector compared to *Ae. aegypti* in the tropics.

3.3. Maintenance and Stability of Interhuman Transmission, and Future Prospects

In past centuries, CHIKV introductions into temperate regions where *Ae. aegypti* was also introduced seasonally but could not survive winters probably did not result in sustained endemic transmission. In the tropics, it is thought that transmission could have persisted longer but was not permanent until the past century, based on the lack of endemic/epidemic strains detected with emergence or divergence estimates from ECSA progenitors earlier than the past century (**Figure 1**). However, recent introductions have resulted in more permanent or endemic, human-amplified circulation. The Asian lineage has persisted from 1958 or earlier to the present. This apparent increase in endemic stability during the past century probably reflects the urbanization of the tropics, combined with major increases in *Ae. aegypti* populations since World War II (45). However, it is unclear if endemic transmission is stable in all regions of Southeast Asia; phylogenetic studies and recent Asian lineage CHIKV detection suggest that transmission may be more stable in Indonesia and the Philippines than further north in Malaysia and Thailand (**Figure 1**).

Unfortunately, with the continuation of urbanization trends accompanied by growing populations of *Ae. aegypti*, there is no reason to believe that CHIKV will not become permanently endemic in many parts of the Americas. However, in Europe and North America, even *Ae. albopictus*–adapted CHIKV strains may not be transmitted stably because winter temperatures result in mosquito population declines and reduced transmission due to inefficient arboviral replication in exothermic mosquitoes (60), combined with lower levels of vector–human contact year-round due to cultural differences such as the greater use of air conditioning and window screens, which has been reported to limit dengue in the United States (97). However, in less developed regions of the world without these cultural protections, climate change will allow *Ae. aegypti* to expand its distribution and likely increase the risk for CHIKV epidemics (140).

SUMMARY POINTS

1. CHIKV is a mosquito-borne arbovirus that causes severe, debilitating, and often chronic arthralgia during human-amplified outbreaks.

2. Although CHIKV has caused urban outbreaks for centuries after being spread on sailing ships, a series of particularly explosive outbreaks began in 2005, affecting tens of millions of persons after spread via human air travelers initiated transmission in Asia, the South Pacific, and the Americas; smaller outbreaks occurred in Europe and Africa.
3. CHIKV originates from enzootic, sylvatic transmission cycles in sub-Saharan Africa involving diverse, arboreal mosquito vectors and many species of NHPs, and possibly other mammals, as amplification hosts.
4. Spillover infections of humans (from enzootic hosts to humans via enzootic or bridge vectors) occur within forests, but human amplification probably requires travel to sites distant from enzootic habitats where peridomestic vector and human densities are adequate.
5. Although *Ae. aegypti* is the principal CHIKV vector owing to its superior vectorial capacity, recent outbreaks have exploited the dramatic invasion of Africa, Europe, and the Americas by *Ae. albopictus*.
6. A series of *Ae. albopictus*–adaptive mutations in CHIKV strains of the IOL have facilitated transmission in more rural and temperate regions, allowing the virus to expand its geographic range.
7. Epistatic limitations on these vector-adaptive mutations, as well as major variation in CHIKV virulence and transmissibility, have resulted from founder effects that probably accompany point-source introductions, involving viral population bottlenecks, associated with infection of mosquitoes by human travelers, as well as occurring during transmission via viremic mosquito saliva.

DISCLOSURE STATEMENT

S.C.W. holds patents for the development of chikungunya vaccines. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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