HHS Public Access

Author manuscript

Curr Opin Virol. Author manuscript; available in PMC 2017 March 14.

Published in final edited form as:

Curr Opin Virol. 2016 February ; 16: 143–150. doi:10.1016/j.coviro.2016.02.007.

Interspecies transmission and chikungunya virus emergence

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Abstract

Chikungunya virus (CHIKV) causes severe, debilitating, often chronic arthralgia with high attack rates, resulting in severe morbidity and economic costs to affected communities. Since its first well-documented emergence in Asia in the 1950s, CHIKV has infected millions and, since 2007, has spread widely, probably via viremic travelers, to initiate urban transmission in Europe, the South Pacific, and the Americas. Some spread has been facilitated by adaptive envelope glycoprotein substitutions that enhance transmission by the new vector, *Aedes albopictus*. Although epistatic constraints may prevent the impact of these mutations in Asian strains now circulating in the Americas, as well as in African CHIKV strains imported into Brazil last year, these constraints could eventually be overcome over time to increase the transmission by *A. albopictus* in rural and temperate regions. Another major determinant of CHIKV endemic stability in the Americas will be its ability to spill back into an enzootic cycle involving sylvatic vectors and nonhuman primates, an opportunity exploited by yellow fever virus but apparently not by dengue viruses.

Introduction

Emergence history of chikungunya virus Chikungunya virus

(CHIKV), an alphavirus in the family *Togaviridae*, causes severe, debilitating and often chronic arthralgia. First discovered during a 1952–1953 outbreak in present day Tanzania, this mosquito-borne virus has since been implicated in explosive epidemics involving millions of persons in Africa, Asia, Europe, the South Pacific, and most recently the Americas [1,2]. Like all arthropod-borne viruses (arboviruses), CHIKV is zoonotic, and evolved in enzootic transmission cycles in sub-Saharan Africa involving forest-dwelling mosquito vectors and nonhuman primate (NHP) amplification hosts. Initial phylogenetic studies identified two major enzootic lineages: West African and East/Central/South African

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(ECSA) (Figure 1) [3]. An Asian lineage, first isolated in 1958 and implicated in outbreaks in India and South-east Asia during the 1960s, was inferred phylogenetically to have been derived from the ECSA lineage, probably following its introduction into Asia between 1879 and 1956 [4•]. However, historic records [5••,6] suggest that CHIKV had been introduced even earlier into Asia and the Americas via sailing ships, the same mechanism believed to have introduced yellow fever and dengue viruses into port cities during past centuries.

After the 1960s, CHIKV was only occasionally associated with human disease in Africa and Asia, probably due to the lack of available diagnostics and the difficulty in distinguishing human infections from dengue fever and other tropical, acute febrile diseases. However, an explosive outbreak that began in coastal Kenya in 2004 and spread first into the Indian Ocean basin, then independently into the Indian Subcontinent followed by South-east Asia (Figures 1 and 2), generated renewed attention to this reemerging virus. As millions of persons became infected, thousands of air travelers introduced this new Indian Ocean Lineage (IOL) of CHIKV, also derived from an ECSA enzootic strain (Figure 1), into nearly all regions globally, and local transmission ensued in northern Italy [7] and southern France (Figure 2) [8,9]. Al-though importations were also documented in transmission-permissive regions of the Americas, autochthonous cases were not detected in the Western Hemisphere until late 2013 on the Island of St. Martin in the Caribbean [10,11]. Subsequently, CHIKV spread to nearly all Caribbean Islands and into northern South America, Central America, and Mexico. Eleven autochthonous cases were detected in 2014 in Florida, although there was no evidence of persistence. Surprisingly, this first CHIKV strain identified in the Americas was not the IOL lineage implicated in most recent outbreaks, but belonged to the Asian lineage that had been circulating in the urban cycle at least since the 1950s and which has continued to cause outbreaks in Asia and Oceania in recent years [12,13]; importation probably occurred via a viremic traveler from Southeast Asia or Oceania, or possibly an infected mosquito transported on a flight (Figure 1) [14••].

Following the dramatic introduction of CHIKV into the Americas in late 2013, additional transcontinental transfers continued. In June, 2014, a traveler from Angola introduced an ECSA strain into Feira de Santana, Brazil (Figure 2) [15••]. This strain could represent a major public health challenge because, like IOL strains, it could adapt for efficient transmission by *Aedes* (*Stegomyia*) *albo-pictus* (details below). Then, in October, 2014, a traveler from Cameroon initiated an outbreak involving 11 con-firmed cases in Montpellier, France, with *A. albopictus* suspected as the principal vector (Figure 2) [16].

With the increased attention on CHIKV since 2005, additional cases and outbreaks have also been detected in Africa, including in Cameroon [17,18], Gabon [19–21], Congo [http://www.ncbi.nlm.nih.gov/pubmed/25541718], Tanzania [22] and Senegal [23]. Some of these infections probably represented spillover from enzootic cycles without a switch to urban vectors, while in others urban mosquitoes such as *A. albopictus* were implicated in inter-human transmission.

Phylogenetic reconstruction of chikungunya virus evolution

The first phylogenetic trees of CHIKV, based on partial E1 glycoprotein gene sequences and the 3′ untranslated genome region (3′UTR), identified two main enzootic lineages: West

African and ECSA [3]. These studies concluded that the Asian Lineage urban strains resulted from an introduction of an ECSA progenitor 50–430 years before 2000. More recent Bayesian coalescent estimates based on complete open reading frame sequences are 1879–1956 (Figure 1) [4•]. This study also determined that the IOL strains, which appeared in Coastal Kenya in 2004 [24], spread independently into islands in the Indian Ocean and into the Indian subcontinent in 2005. Although the IOL has not been detected in the Indian Ocean islands since 2011, it continues to circulate and evolve in South and Southeast Asia, with small outbreaks exported to Italy and France as described above. IOL divergence has included several E2 envelope glycoprotein substitutions that further enhance infectivity for *A. albopictus*, as discussed below (Table 1). Since the first 2013 Caribbean outbreak, Asian CHIKV strains remain highly conserved, consistent with a point source introduction, and with no evidence of adaptive evolution (Figure 1). Finally, phylogenetic placement as sisters to a 1962 Angola ECSA CHIKV strain of three isolates from Feira de Santana, Brazil (Figure 1) support the importation of this strain by a traveler from southern Africa [15••].

Enzootic chikungunya virus transmission

Enzootic cycles of CHIKV in sub-Saharan Africa are believed to represent its ancestral state (Figure 1). There, in forested habitats, CHIKV transmission has been documented involving several mosquito species in the genus *Aedes*, with nonhuman primates and possibly other vertebrates serving as amplification hosts (Figure 3). *Aedes* (*Diceromyia*) *furcifer* appears to be the most important enzootic vector in South Africa [25], Zimbabwe [26] and Senegal [27], while *A.* (*Diceromyia*) *taylori*, *A.* (*Stegomyia*) *africanus* [28] and *A.* (*Stegomyia*) *luteocephalus* have also been implicated on multiple occasions [27,29]. Of these vectors, the susceptibility to infection has only been determined experimentally for *A. furcifer*, which transmitted CHIKV between African green monkeys (*Cercopithecus aethiops*) and exhibited an oral infection threshold of less than $10^{4.5}$ log infectious units.

The role of various NHPs in enzootic CHIKV amplification has received limited attention. African green monkeys and chacma baboons (*Papio ursinus*) were suggested as important hosts in Zimbabwe based on high seroprevalence [26], and the former species developed viremia after experimental infection in Uganda [28]. In an enzootic Senegal focus, CHIKV was isolated from African green and Patas (*Erythrocebus patas*) monkeys and from the Guinea baboon (*Papio papio*) [27,30]. Chimpanzees were also seropositive in the Congo [31]. While a variety of other wild and domesticated vertebrates have been found seropositive in Africa and Asia, antibody titers are generally low and few experimental infections have been performed. Overall, these data suggest that NHPs are important amplification hosts but do not rule out a role for other vertebrates in enzootic maintenance.

In Asia, there is no conclusive evidence of enzootic CHIKV circulation. In Peninsular Malaysia with its history of repeated CHIKV outbreaks, 71 Bornean orangutans [32] were seronegative, and only one of 147 long-tailed macaques (*Macaca fascicularis*) was positive [33]. Regardless, temporary spillback of urban strains cannot be ruled out as an explanation of this or other NHP infections. Isolation of CHIKV strains genetically distinct from urban isolates and/or detection of circulation in regions remote from humans would be needed to

demonstrate an independent enzootic cycle. This would be an important finding that might explain CHIKV persistence during interepidemic periods.

In summary, CHIKV appears to use several different NHPs and *Aedes* spp. vectors for enzootic circulation in Africa. This pattern of opportunistic vertebrate host and mosquito vectors usage has been described for other alphaviruses [34], as well as other arboviruses [35]. Flexibility in primate usage is probably critical due to the limited population sizes and turnover rates for these long-lived hosts. Furthermore, this flexibility in host and vector usage is an important factor in the ability of CHIKV to emerge via host range changes, as described below.

Vector and primate host range changes associated with urban chikungunya virus emergence

The urban CHIKV cycle presumably commences when a person entering or living near African forests becomes infected via enzootic spillover due to vectors such as *A. furcifer*, which enters villages and bites people (Figure 3) [23]. In Africa, the role of *A.* (*Stegomyia*) *aegypti* in CHIKV transmission is far from clear; the domesticated subspecies (*A. aegypti aegypti*) evolved convergently from the ancestral, zoophilic form (*A. aegypti formosus*) by acquiring a preference for artificial water containers as oviposition sites and humans as a blood and nutrition source [36]. Human migration led to the establishment of this sub-species in most tropical and subtropical regions of the world, allowing it to serve as the main global CHIKV vector [37]. Similar to *A. aegypti* but more recently, *A. albopictus* extended its range from Asia [38] into tropical and temperate zones of the Americas, Africa and Europe [39–43]. However, until recently the role of this species in CHIKV transmission was thought to be less important compared to *A. aegypti*, mainly due to its less prominent anthropophily. Although the current distributions in many parts of Africa of *A. albopictus* and the domesticated form of *A. aegypti* remain incompletely characterized, presumably a person infected from enzootic CHIKV spillover occasionally reaches a location where populations of these mosquitoes and their contact with people are sufficient to initiate interhuman transmission.

There is no direct evidence of any adaptive constraints on the ability of enzootic CHIKV strains to enter the urban cycle (Figure 3). However, studies of the *A. albopictus*-adaptive substitution E1-A226V (Table 1) suggest that acquisition of the E2-I211T substitution in ECSA strains could increase CHIKV fitness for human infection [44•]. This conclusion is based on the fact that the E2-211I residue is the most common among the enzootic ECSA strains, including those ancestral to the IOL [44•,45]; however strains involved in continuous interhuman circulation, including the IOL and Asian lineages, exhibit E2-I211T. Experimental studies failed to support a phenotype for E2-I211T (in the absence of E1-226V) in CHIKV infection of urban vectors, leaving adaptation to humans as a possible explanation for its convergent evolution before E1-A226V [44•].

In contrast to *A. aegypti*-borne transmission, urban IOL outbreaks involving interhuman transmission by *A. albo-pictus* have been consistently associated with an alanine-to-valine substitution at position 226 of the E1 glycoprotein (Table 1), including at least 6 documented occasions: Reunión island, 2006 [46••], in Indian states of Kerala, 2007 [47–49] 2009 [50],

twice in Sri Lanka, 2008 [51], and once in Gabon, 2007 [52,53]. This convergent evolution strongly suggests adaptation for enhanced *A. albopictus* transmission. Using natural CHIKV isolates from the 2006 Reunión outbreak differing only in this residue, Vazeille *et al.* demonstrated that the presence of E1-226V is associated with enhanced CHIKV dissemination and higher viral RNA loads in *A. albopictus* [54•]. Reverse genetic experiments demonstrated that E1-A226V results in ~40–100-fold increase in CHIKV oral infectivity to *A. albopictus* and mediates more efficient virus transmission to mice [44•,55•, 56,57••]. Subsequent studies using single-round infectious CHIKV-like particles [56] and intra-thoracically injected *A. albopictus* [58,59] demonstrated that E1-A226V acts primarily by increasing CHIKV oral infectivity for midgut epithelial cells, which leads to more efficient transmission.

Further research demonstrated that, in addition to E1-A226V, most *A. albopictus*-borne CHIKV strains have acquired additional, second-step substitutions located in the Acid Sensitive Region (ASR) of the adjacent E2 glycoprotein. This E2 region mediates a conformational change in the E2/E1 heterodimer within low pH endosomes to expose the E1 fusion peptide for entry into the cytoplasm (Table 1) [56,60••]. These substitutions appeared after acquisition of E1-A226V and have a lesser but still highly significant (typically resulting in a 5–16-fold increase in CHIKV infectivity) effect on CHIKV infection of *A. albopictus*, compared to the E1-A226V. The ability of CHIKV to use several different second-step adaptive mutations to more efficiently infect *A. albopictus* may have facilitated the rapid IOL diversification.

A critical finding related to the E1-A226V and its role in CHIKV emergence was that the valine residue had never been detected in Asian lineage CHIKV strains despite their long history of circulation in regions native to *A. albopictus* [4•]. An explanation for this conundrum, that not all CHIKV strains are equally susceptible to the effects of the E1-A226V substitution, was supported by reverse genetic studies. The E1-98T residue, found in all Asian strain sequences but absent from all other CHIKV lineages, completely blocks the E1-226V-enhanced oral infection phenotype in *A. albopictus* (Table 1) [57••], and thereby apparently prevented Asian CHIKV strains from adapting to this vector. This, in turn, may have facilitated the invasion of *A. albopictus*-adapted IOL strains into Southeast Asia beginning in 2006 to exploit this unoccupied vector niche [57••]. Like E1-98T, the E2-211I residue found in most ECSA strains (including the independent introduction into Feira de Santana, Brazil) also limits penetrance of E1-226V and thus CHIKV's potential to emerge in some parts of Africa and the Americas via *A. albopictus* transmission [44•]. It remains to be seen if ECSA strains in Brazil will select for E2-I211T, as did early IOL strains in Kenya in 2004 [45], which will enable subsequent *A. albopictus*-adaptive evolution.

Prospects for expansion of enzootic chikungunya virus host range and distribution

The information summarized above may be useful in predicting the outcome of current and future CHIK outbreaks. Its historic evidence of repeated reemergence combined with the lack of evidence for adaptive barriers to initiation of the urban transmission cycle by enzootic progenitors strongly suggests that CHIKV will continue to emerge periodically and indefinitely from Africa to initiate epidemics. The ECSA origin of the past two emergences

(Asian and IOL) may only reflect the geo-graphic proximity of these strains to Asia, which is highly permissive to urban circulation; but there is no evidence that West African strains are incapable of initiating inter-human transmission. Furthermore, based on our current understanding, the genomic sequences of many ECSA and West African strains may be fully capable of adapting for efficient *A. albopictus* transmission, as has been seen in IOL strains (Table 1). However, epistatic constraints in Asian strains [57••], which are now widespread in the Americas, and in the ECSA strain introduced last year into Brazil, suggest that these lineages may remain primarily transmitted by *A. aegypti* with less opportunity for movement into temperate regions where this mosquito cannot survive cold winters. The initial incrimination of *A. aegypti* in transmission in the region of the Americas inhabited by both urban vectors (Mexico) [61] supports this prediction. Opportunities for the use of additional urban vectors are difficult to predict in the absence of experimental data, but recent evidence that *Aedes* (*Stegomyia*) *hensilli* was the principal epidemic CHIKV vector on Yap Island suggests that other mosquitoes may play major roles in some regions [12].

Similarly, there is no direct evidence for a barrier to CHIKV host jumps from African NHPs into humans that accompany urban emergence. As discussed above, CHIKV may not specialize on any given NHP species and thus the use of humans may be consistent with a generalized competence for amplification in many different primates. If this is the case, CHIKV may have the potential to spill back into an enzootic South American cycle, as occurred for yellow fever virus after its importation from Africa during the slave trade [62]. However, the lack of evidence for such CHIKV spillback in Asia despite many decades if not centuries of opportunity may reflect an unrecognized barrier to the use of NHPs or sylvatic mosquito vectors in some geographic regions. The apparent failure of dengue viruses to establish enzootic trans-mission in the Americas [63] suggests such a barrier. Experimental infections of New World primates and sylvatic, primatophilic mosquitoes as well as incorporation of sylvatic regions into CHIKV surveillance will be important for determining the potential for enzootic establishment, which could stabilize CHIKV endemicity in the Americas and ensure continuous risk.

Acknowledgments

This research was supported by NIH grant AI069145 and by grant HSHQDC-13-C-B0009, from the Department of Homeland Security entitled ‘Whole Genome Approach to Microbial Forensics Technical Focus Area-3.’

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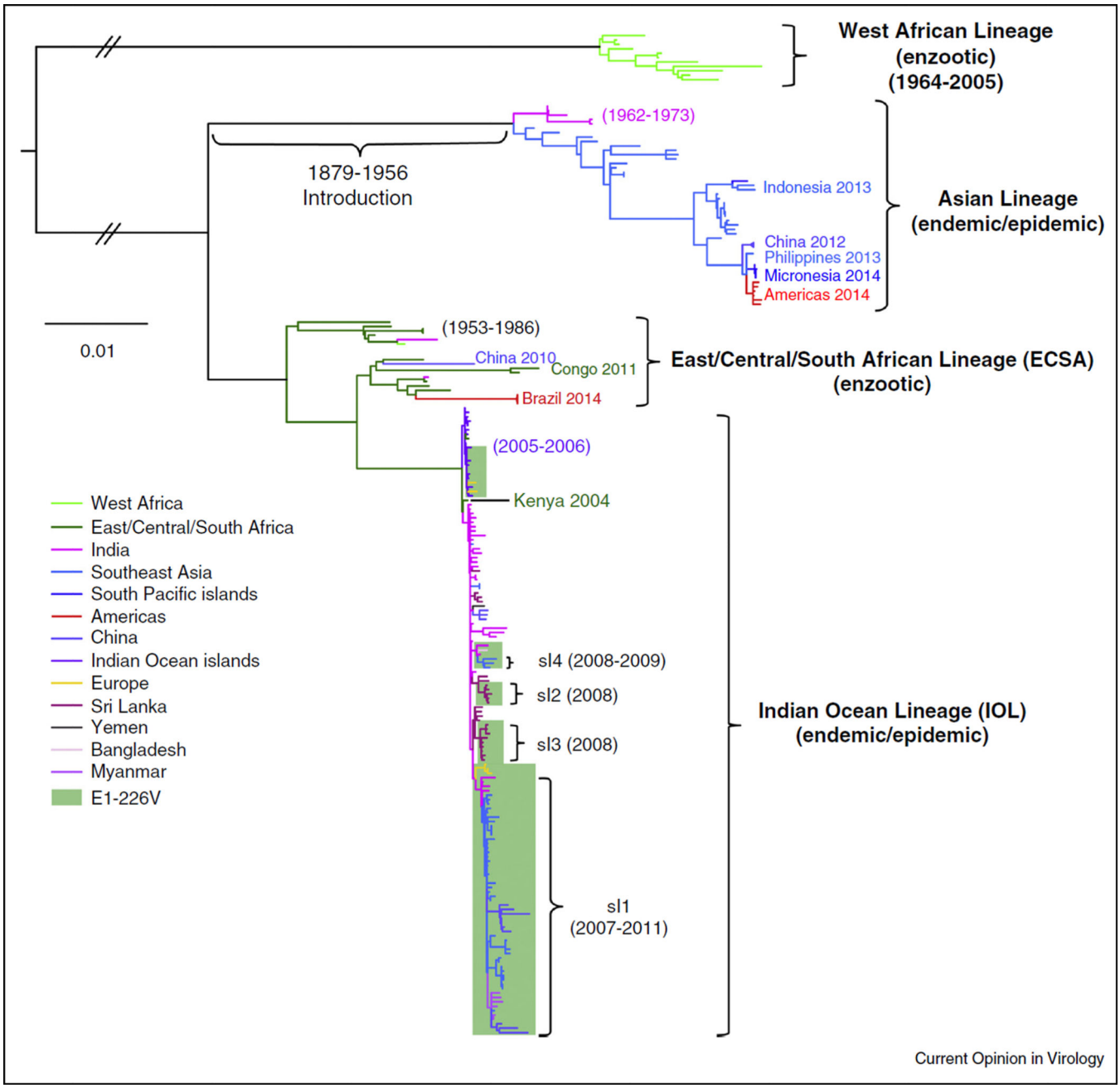

Figure 1.

Phylogenetic tree showing the relationships among enzootic and endemic/epidemic CHIKV lineages and a time estimate for introduction of the Asian lineage from Africa into Asia; sl indicates IOL sublineage with second-step *A. albopictus*-adaptive mutations [56]. The tree was generated using concatenated open reading frames from all complete genomic sequences found in the Genbank library using maximum likelihood implemented in PAUP 4.0 [64]. The branch colors represent countries or regions of origin for each CHIKV sample. The scale indicates nucleotide sequence divergence.

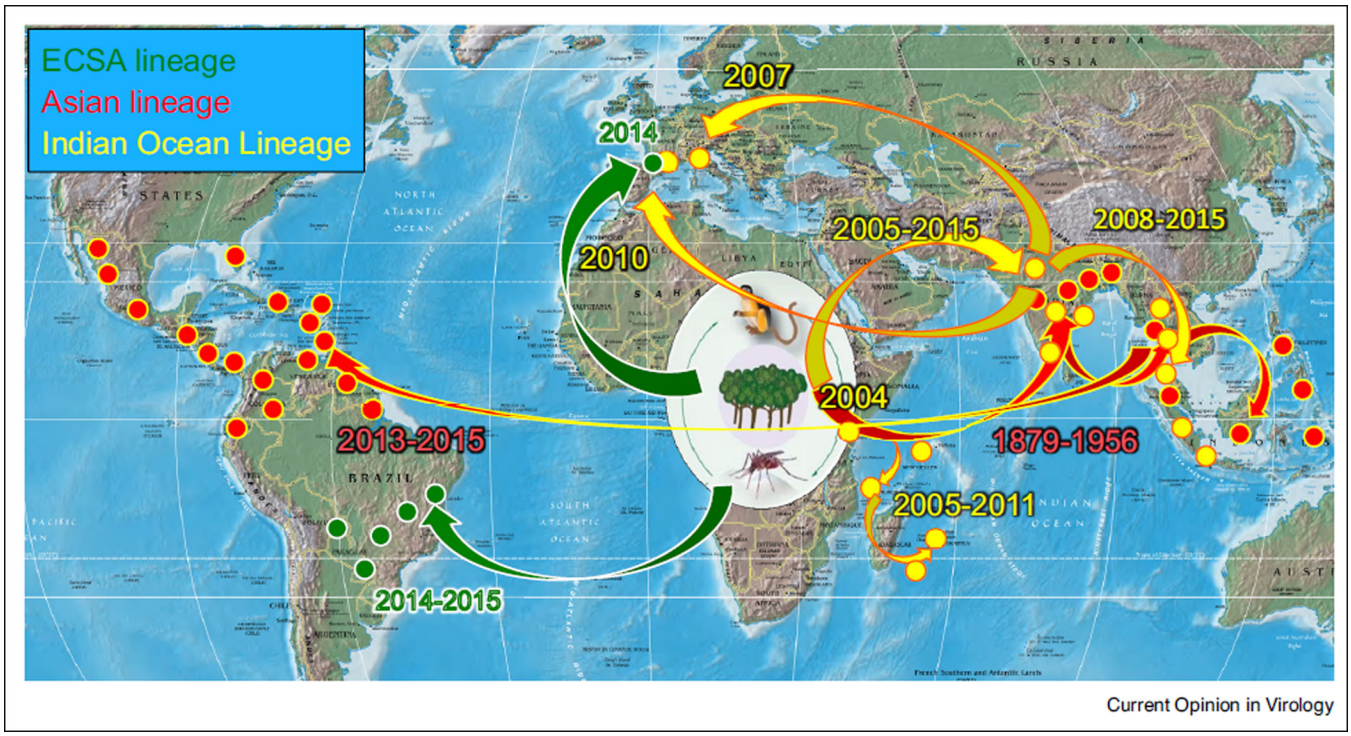

Figure 2.

Map showing the historic spread of CHIKV from enzootic cycles in Africa to Asia, Europe and the Americas.

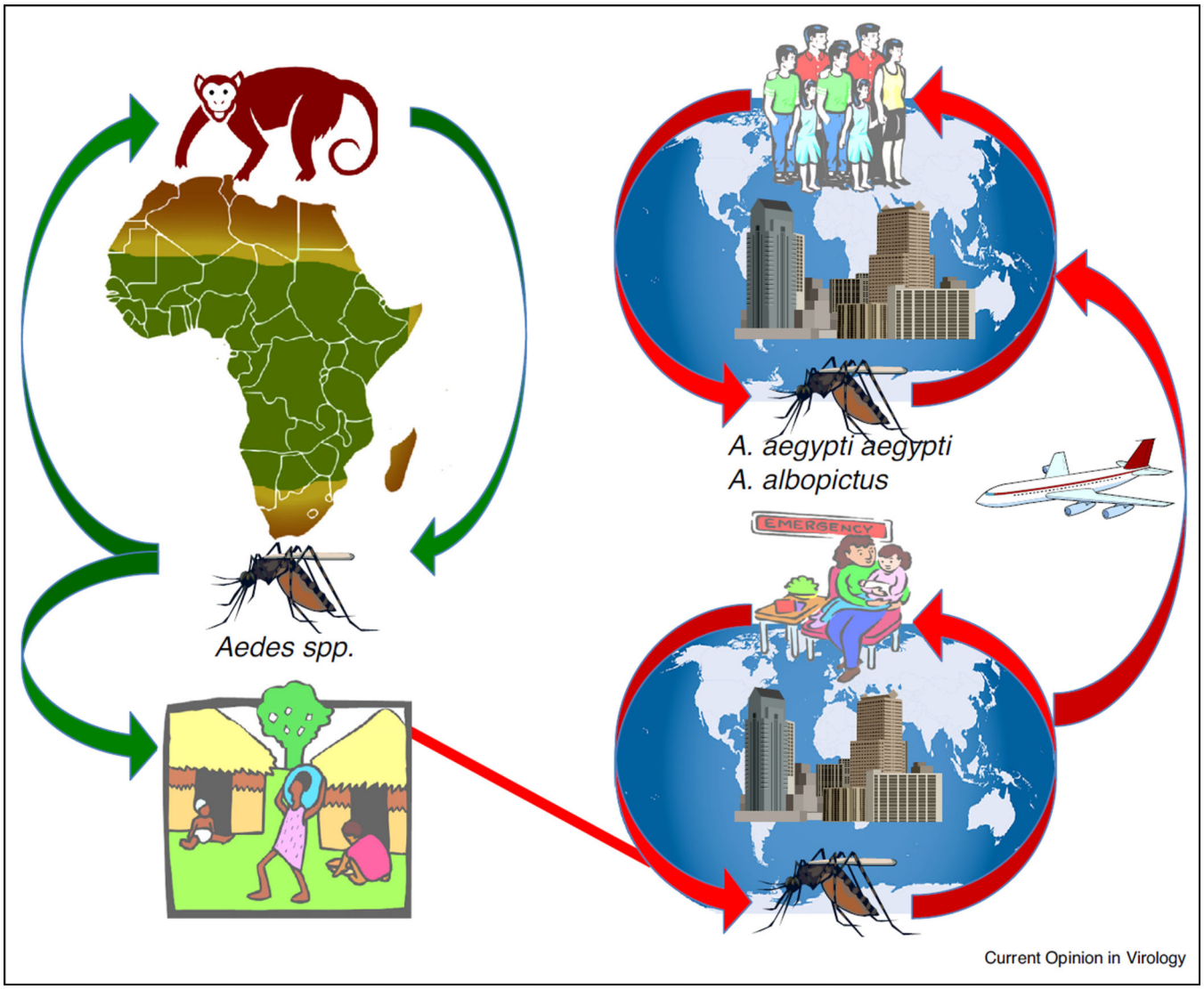

Figure 3.

Cartoon showing enzootic and urban CHIKV transmission cycles and their connection in sub-Saharan Africa.

Table 1

Amino acid substitutions that influence *Aedes albopictus* infection by CHIKV

CHIKV Lineage	Year of first appearance	Protein	Amino acid substitution (references)	Approximate infectivity increase or epistatic effect	Epistatic interactions
IOL	2005	E1	A226V [55•,56,60••]	40-fold	E1-98, E2-211
IOL SL1	2007	E2	K252Q [60••]	8-fold	ND
IOL SL2 (partial)	2008	E2	K233E [60••]	6-fold	ND
IOL SL3B	2008	E2/E3	R198Q/S18F (synergistic)[60••]	16-fold	ND
IOL SL4	2009	E2	L210Q [56]	5-fold	ND
Asian	1958	E1	A98T [57••]	Completely prevents penetrance for *A. albopictus* infection	E1-226V
ECSA	1953	E2	I211T [44•]	Enables penetrance for *A. albopictus* infection	E1-226V

ND — not determined; SL — sublineage.