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Chikungunya: Evolutionary history and recent epidemic spread

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ABSTRACT

Chikungunya virus (CHIKV) has a long history of emergence into urban transmission cycles from its ancestral, enzootic, sylvatic foci in Sub-Saharan Africa, most recently spreading to the Americas beginning in 2013. Since 2004, reemergence has resulted in millions of cases of severe, debilitating and often chronic arthralgia on five continents. Here, we review this history based on phylogenetic studies, and discuss probable future spread and disease in the Americas. We also discuss a series of mutations in the recently emerged Indian Ocean Lineage that has adapted the virus for transmission for the first time by the *Aedes albopictus* urban mosquito vector, and compare CHIKV to other arboviruses with and without similar histories of urbanization. This article forms part of a symposium in *Antiviral Research* on "Chikungunya discovers the New World."

Contents

1. The discovery of Chikungunya in East Africa

Chikungunya fever (CHIKF), an acute febrile disease typically accompanied by rash and severe, debilitating and often chronic arthralgia, has probably occurred in Africa for centuries or longer, with exported outbreaks in Asia and the Americas during the 18th and 19th centuries (Halstead, 2015; Carey, 1971). In fact, Halstead has argued that the disease originally described as dengue was actually CHIKF. The first outbreak was recognized during the modern scientific era in July, 1952 when an epidemic occurred along the coastal plateaus of Mawia, Makonde and Rondo in present day Tanzania (Lumsden, 1955). The low incidence of malaria in this region may have facilitated the recognition of CHIKF, which was described as a "very sharp onset of crippling joint pains, severe fever, and eventually the conspicuous rash" (Ross, 1956).

Attack rates in the epidemic region averaged approximately 40–50% in various villages. The soil of these plateaus is highly permeable, requiring local residents to store water in their villages. This resulted in large populations of *Aedes* (*Stegomyia*) *aegypti*, "including a considerable portion of the pale form" (Lumsden, 1955) generally considered to be the domesticated form *A. aegypti*

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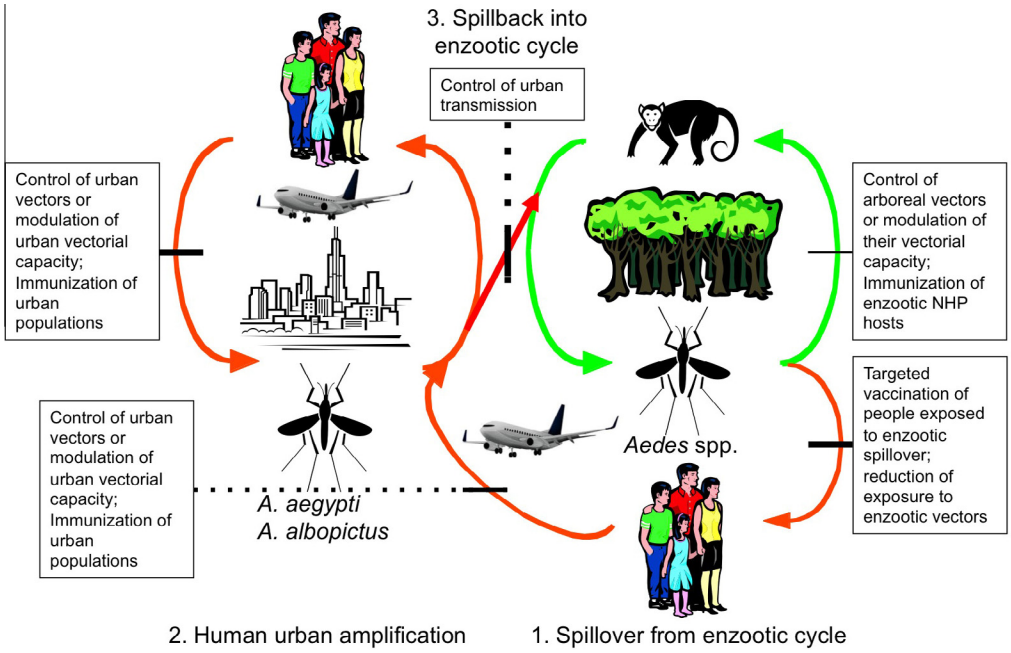

Fig. 1. Enzootic (right) and epidemic (left) transmission cycles of CHIKV. Epidemics are believed to result from spillover enzootic infections to persons working in forested habitats or living in villages nearby, followed by transport to urban centers populated by the epidemic vectors *A. aegypti* and/or *A. albopictus*. Additional spread occurs readily by infected air travelers who typically are in the 1–4-day asymptomatic period of incubation. Lines through arrows indicate potential points of intervention of enzootic circulation, spillover infections of humans, introductions into the urban cycle, and spillback from urban cycles to initiate arboreal, enzootic cycles. Thickness of black lines reflect the likelihood of success of these interventions (thicker = more likelihood of success). Adapted from Weaver (2013), with permission.

aegypti that efficiently transmits arboviruses such as yellow fever and dengue (Powell and Tabachnick, 2013). Two pools of this species and one of *Culex* spp. yielded virus isolates, and the feeding of laboratory-reared *A. aegypti* on febrile patients followed by extrinsic incubation and feeding on mice resulted in lethal murine infections (Ross, 1956). The virus, Chikungunya virus (CHIKV) was derived from a descriptive term applied by the local Makonde people, which can be roughly translated as "the disease that bends up the joints" (Ross, 1956). Although CHIKV was first thought to be a strain of dengue virus (Ross, 1956), it was later shown to be a Group A virus, which eventually was included in the genus *Alphavirus* (Calisher and Karabatsos, 1988).

Soon after its discovery in East Africa, human infections were detected in Uganda (Weinbren, 1958) and the arboreal mosquito, *A. africanus*, was found to be naturally infected (Weinbren, 1958). This was the first evidence of a sylvatic, enzootic CHIKV cycle. Subsequently, CHIKV was discovered in many parts of sub-Saharan Africa, with transmission mainly by arboreal mosquitoes (Fig. 1) [reviewed in (Coffey et al., 2014)]. Later, in 1964, antibodies were detected in nonhuman primates (NHPs) captured in current Zimbabwe, and vervet monkeys were shown to be competent amplification hosts for CHIKV using mosquito transmission (Paterson and McIntosh, 1964). Several studies since that time have corroborated the roles of NHPs as amplification hosts and of *A. africanus*, *A. furcifer* and other arboreal mosquitoes as enzootic vectors [reviewed in (Coffey et al., 2015)]. Interestingly, only recently was evidence of human CHIKV infections in Tanzania obtained again (Hertz et al., 2012). Eventually, initial (Powers et al., 2000) and more recent (Volk et al., 2010) sequencing and phylogenetic studies placed representative CHIKV strains from all of these sub-Saharan African locations into one clade termed the East/Central/South American lineage (Fig. 2).

The later part of the 20th century saw expanded recognition of enzootic CHIKV in many parts of sub-Saharan Africa, along with occasional, sporadic outbreaks of CHIKF in Africa and Asia. Longitudinal studies of enzootic CHIKV in eastern Senegal revealed a pattern of periodic, major amplification, detected by infection of enzootic vectors, in 1975, 1979, 1983, and 1992 (Diallo et al., 1999, 2012). CHIKV isolates from these and a few other studies in the region were eventually sequenced and shown to comprise a second enzootic clade termed the West African lineage (Fig. 2) (Volk et al., 2010; Powers et al., 2000). Whether this pattern of amplification described in Senegal represents local extinctions and reintroductions of CHIKV, or stable enzootic circulation accompanied by periodic amplification in NHPs, remains unknown. Although seroprevalence in Senegalese NHPs as well as virus isolations indicate their probable role as amplifying hosts, the broad host ranges of the enzootic vectors there suggest that other vertebrates could also serve as reservoir hosts (Diallo et al., 2013). Additional serosurveillance in enzootic regions of Africa combined with experimental infections of wild vertebrates is needed to further define the critical hosts for enzootic circulation. This information, along with the assessment of potential arboreal mosquito vectors and native as well as introduced NHPs (e.g. African green monkeys, enzootic African hosts that were introduced to several Caribbean Islands), is especially important in assessing the risk for the development of enzootic cycles in regions of new introductions such as the Caribbean and Latin America.

2. Chikungunya outbreaks in Asia before 2004

The first direct evidence of CHIKF outside of Africa came from Bangkok, Thailand, where CHIKV was first isolated in 1958 during an outbreak associated with *A. aegypti* transmission (Figs. 2 and 3) (Hammon et al., 1960). Subsequently, CHIKV was also discovered in Cambodia and India from 1961 to 1963, associated with outbreaks that were often mixed with dengue and usually associated with *A. aegypti*. Immunity to Semliki Forest virus described in Bangkok in 1957 [cited in (Hammon et al., 1960)] suggested that CHIKV (closely related and antigenically cross-reactive with Semliki Forest) had been present previously. CHIKV antibodies were also detected in Indian sera collected as early as 1954

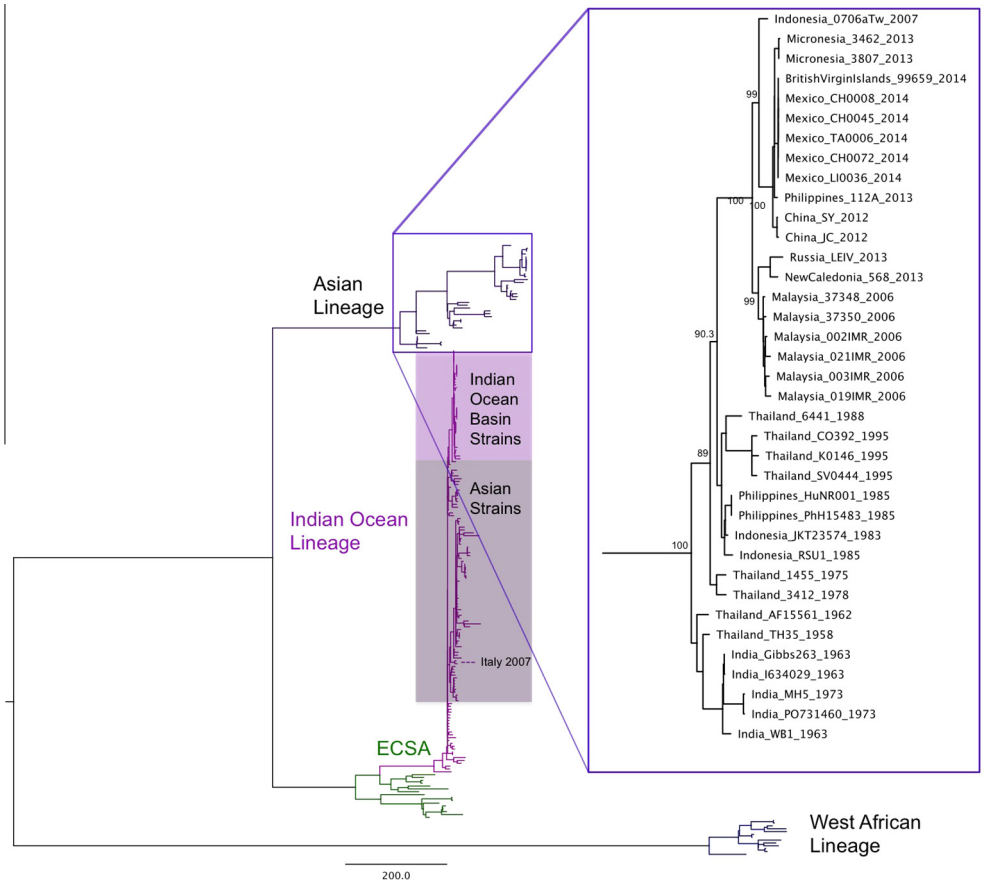

Fig. 2. Phylogenetic tree showing relationships among chikungunya virus strains and major lineages derived from complete genomic sequences using neighbor-joining methods. The topology of the inset tree is identical to that constructed using Maximum Likelihood and Bayesian methods (Ronquist and Huelsenbeck, 2003). Bootstraps were generated using 1000 replicates and shown only on major branches for clarity. ECSA, East, South, Central African enzootic lineage.

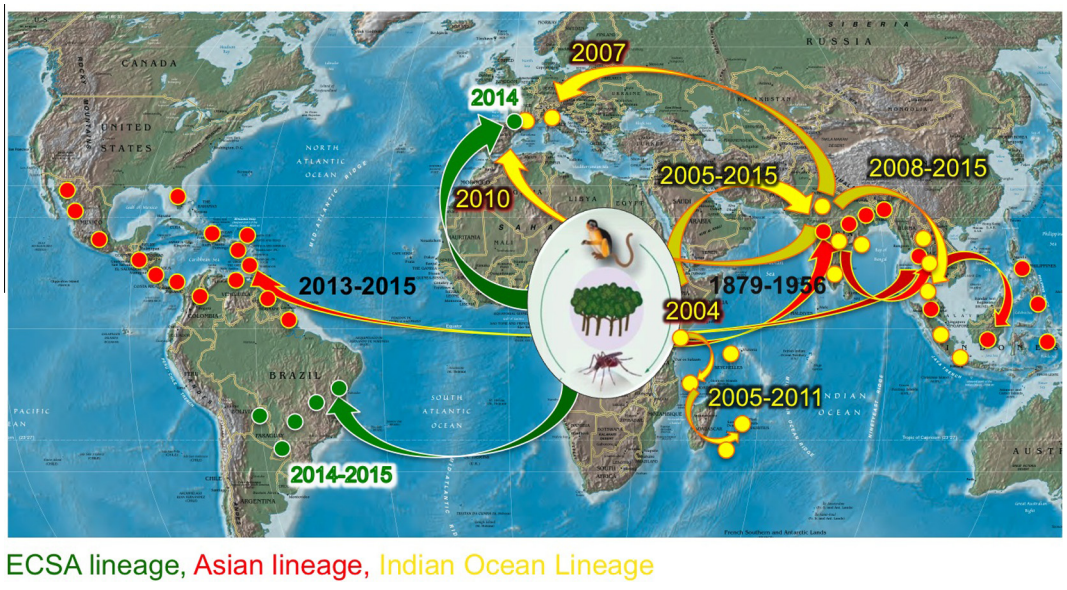

Fig. 3. Map showing the known historic spread of Chikungunya virus based on phylogenetic reconstructions (Lanciotti and Valadere, 2014; Volk et al., 2010; Tsetsarkin et al., 2014, 2011b), as well as recent introductions (http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18f-f4cb1b568&ID=1096) Nunes et al. (2015). Green dots, arrows and years indicate the East/Central/South African (ECSA) lineage, Red dots, arrows and years indicate the Asian lineage, and yellow dots, arrows and years indicate the Indian Ocean lineage (IOL).

(Pavri, 1964). Higher rates of CHIKV seroprevalence and lower attack rates among persons over 50 years of age in Sri Lanka also suggested that the virus had circulated there during the early 20th century (Hermon, 1967).

An even earlier presence of CHIKV in Asia was suggested by Carey (Carey, 1971), who made a strong case that an outbreak of 'knokkel korts,' or "knuckle/joint fever" described in Batavia (present-day Jakarta) in 1779 was an outbreak of CHIKF. This

interpretation is plausible because yellow fever and dengue viruses, with identical epidemic transmission cycles involving *A. aegypti* and humans, are known to have been disseminated aboard sailing ships during this era to initiate epidemic transmission in many port cities.

Initial (Powers et al., 2000) and more recent (Volk et al., 2010) sequencing and phylogenetic studies demonstrated that CHIKV strains isolated during the Asian outbreaks from 1958 to 1973 comprise a monophyletic group placed basal to the East, Central, South Africa (ECSA) lineage, now termed the Asian epidemic lineage (Fig. 2). Coalescent estimates indicate that the Asian strain diverged from an enzootic ECSA strain between 1879 and 1956, inconsistent with the presence of the same lineage in Indonesia in 1779, and has continued to circulate in Asia ever since.

The Asian CHIKV lineage apparently became extinct in India after 1973 but continued to circulate in Southeast Asia, occasionally detected during small-medium sized epidemics. Although the presence of antibodies in Asian NHPs (Wolfe et al., 2001) suggested the possibility of an enzootic cycle there (in addition to Africa), spillback from the urban cycle (Fig. 1) cannot be ruled out as an alternative explanation.

3. Emergence and spread of the novel epidemic IOL strain

The history of CHIKF took a dramatic turn in 2004 when a new epidemic strain emerged from the ECSA enzootic lineage. First detected circulating in coastal Kenya (Chretien et al., 2007; Kariuki Njenga et al., 2008), CHIKV spread in 2005 into islands in the Indian Ocean, where major outbreaks with high attack rates occurred (Fig. 3). The best studied, on the French Island of La Réunion, involved about 300,000 cases with high rates of apparent infection and an overall attack rate of approximately 1/3 (Schilte et al., 2013; Gerardin et al., 2008).

During the course of the Réunion epidemic, genomic sequencing of CHIKV isolates revealed a transition in the predominant amino acid at position 226 of the E1 envelope glycoprotein from Ala to Val (Fig. 4) (Schuffenecker et al., 2006). Because this residue was implicated previously in the ability of SFV to infect (*A. Stegomyia*) *albopictus* mosquito cells, and because the principal epidemic vector in past outbreaks, *A. aegypti* was uncommon on this island, it was hypothesized that this E1 substitution might be involved in the use of the more common *A. albopictus* for transmission there. *A. albopictus* was known to be highly susceptible to CHIKV infection (Shah et al., 1964) but had never been implicated as a major vector. Experimental infections supported the adaptation hypothesis, with the E1-A226V substitution conferring a major increase in the ability of La Réunion CHIKV strains to infect *A. albopictus* despite little effect on infection of *A. aegypti* (Tsetsarkin et al., 2007; Vazeille et al., 2007). This hypothesis that E1-A226V was selected by *A. albopictus* was further supported by the convergent evolution of this substitution in several areas of Asia and Africa where this mosquito is prevalent (Volk et al., 2010; de Lamballerie et al., 2008). The mechanisms of this vector-adaptive mutation and those of others are discussed in more detail in text Box 1.

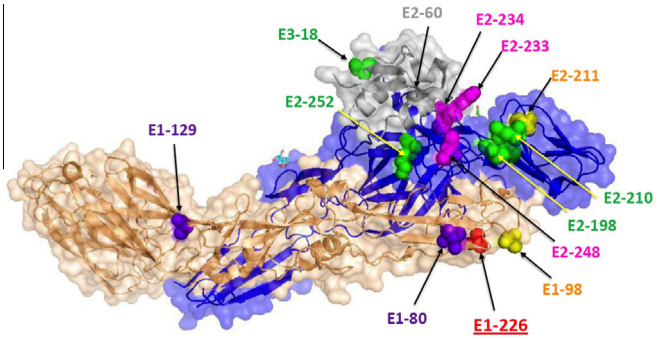

Fig. 4. Map showing envelope glycoprotein substitutions that affect CHIKV fitness for transmission by *A. albopictus*, based on a 3D model of E3/E2/E1 spike. The atomic structure of the CHIKV E3 (gray), E2 (blue) and E1 (gold) glycoprotein complex was generated based on [PDB ID: 3N44 (Voss et al., 2010)]. The position of the first-step *A. albopictus*-adaptive E1-A226V substitution (Tsetsarkin et al., 2007) is indicated by red. Positions of substitutions that epistatically control penetrance of the first-step E1-A226V substitution (Tsetsarkin et al., 2011a, 2009) are indicated by yellow. The position of second-step *A. albopictus*-adaptive substitutions E2-L210Q, E2-R198Q/E3-S18F and E2-K252Q (Tsetsarkin and Weaver, 2011; Tsetsarkin et al., 2014) are indicated by green. The position of artificial substitutions (never reported in natural CHIKV isolates) that in laboratory experiments increase CHIKV transmissibility by *A. albopictus* (Tsetsarkin et al., 2014) are indicated by magenta and violet. Gray shows the position of a non-specific determinant of CHIKV attenuation in *A. albopictus* and *A. aegypti* (Tsetsarkin et al., 2009).

Box 1 Molecular adaptation of the Indian Ocean lineage of Chikungunya virus to *Aedes albopictus*. As discussed in the text, the E1-A226V substitution that was selected convergently as the CHIKV IOL spread into regions of the Indian Ocean and Asia inhabited by *A. albopictus* resulted in a ca. 40–100-fold reduction in the infectious blood meal dose required to infect this mosquito (Fig. 4). More detailed studies using virus-like particles including replicons that express reporter genes demonstrated that the enhanced infectivity was manifested at the initial infection of the mosquito midgut epithelial cells (Tsetsarkin and Weaver, 2011). The location of residue 226 near the fusion peptide that mediates viral entry within the acidic environment of endosomes also suggested that this enhanced midgut cell infection involves fusion rather than initial binding to midgut cell receptors. Similar findings were obtained with the subsequent E2 substitutions, showing their effect on initial infection of midgut cells.

Although these E2 substitutions result in only 4–16-fold increases in infection efficiency of *A. albopictus*, less than the 50–100-fold increase caused by the E1-A226 substitution, reverse genetic studies indicate that combinations of these E2 mutations not yet detected in nature can result in further gains in infectivity, suggesting further adaptation of IOL strains for efficient transmission by this vector (Box 1). These *A. albopictus*-adaptive E2 gene mutations all involve glutamine or glutamic acid substituted for others within the acid sensitive region. This E2 domain is critical for a conformational change in the CHIKV spikes within endosomes at low pH to trigger exposure of the E1 fusion peptide for entry. These findings suggest that the IOL envelope glycoprotein adaptive mutations act to enhance the efficiency of entry into *A. albopictus* midgut cells to initiate infection. More detailed entry studies are needed to test this hypothesis.

Surprisingly, the E1-226V residue has never been detected in Asian lineage CHIKV strains despite their circulation for at least 50 years in the native territory of *A. albopictus*. This conundrum is explained by an epistatic constraint on the ability of Asian lineage CHIKV strains to undergo adaptation via this substitution; an additional substitution, E1-T98A, is needed in Asian strains for the E1-226 residue to exert its phenotype in *A. albopictus* (Tsetsarkin et al., 2011a). The lack of E1-98T in any CHIKV strains from Africa supports the hypothesis that this constraint resulted from a founder effect when the Asian lineage was introduced from eastern Africa. Also, other unique characteristics of the Asian lineage 3′UTR strengthen this conclusion (Chen et al., 2013).

Following its spread from Kenya to South Asia, and later to Southeast Asia, with resulting major epidemics involving millions of persons, the new epidemic Indian Ocean Lineage (IOL) continued to diverge and spread in Asia. Although many IOL strains

circulating in regions with large *A. albopictus* populations gained the E1-A226V mutation, other IOL strains circulating in Asian regions predominated by *A. aegypti* retained E1-226A. Continued sequencing of additional Asian strains revealed that several, representing new sublineages, gained additional *A. albopictus*-adaptive mutations, again with no detectable impact on infection of *A. aegypti* (Tsetsarkin and Weaver, 2011; Tsetsarkin et al., 2014).

During the peak of CHIKF epidemics involving IOL strains in the Indian Ocean Basin and Asia, thousands of infected travelers imported CHIKV into nearly all regions of the world (Fig. 2). These resulted in the initiation of small outbreaks in northern Italy (Rezza et al., 2007) and southern France (Grandadam et al., 2011), both associated with *A. albopictus* transmission. These European outbreaks underscored the risk to temperate regions not typically associated with dengue outbreaks, but susceptible to CHIKV circulation due to its ability to use this vector, which can survive cold winters. During the same timeframe, major epidemics in Southeast Asia associated with the CHIKV IOL suggested that it was displacing the older Asian lineage, which had remained there since the 1950s (Figs. 2 and 3). However, the resurgence of the Asian strains was heralded by recent outbreaks in that same region (AbuBakar et al., 2007) along with several islands in Oceania (Lanciotti and Valadere, 2014; Nhan et al., 2014; Kawashima et al., 2014; Ledermann et al., 2014; Roth et al., 2014).

4. Emergence of CHIKV in the Western Hemisphere

During the initial stages of the Indian Ocean and Asian IOL outbreaks, many infected travelers imported CHIKV into the Americas (Lanciotti et al., 2007), but no local transmission was detected. It was therefore surprising that local circulation in the Americas was not detected until 2013, and that the etiologic strain was an Asian lineage CHIKV strain, apparently imported from Southeast Asia or Oceania (Leparc-Goffart et al., 2014; Lanciotti and Valadere, 2014) (Figs. 2 and 3). After quickly sweeping through the Caribbean following its initial discovery in St. Martin, this Asian CHIKV strain has now spread into all Central American countries, most of South America, and northward into northern Mexico. Local transmission following imported cases also resulted in 11 autochthonous cases in Florida during the summer of 2014. Finally, another surprise occurred in southern France in 2014, in the midst of many CHIKF cases imported from the Caribbean, when an ECSA strain imported from Cameroon was implicated in a small outbreak, again involving *A. albopictus* transmission (http://www.ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?List=8db7286c-fe2d-476c-9133-18ff4cb1b568&ID=1096) (Fig. 1).

5. Origins of CHIKV

Exactly when and where CHIKV originated cannot be answered definitively at this time. The closely related o'nyong-nyong virus (ONNV), which causes a clinically indistinguishable human disease during periodic African outbreaks involving *Anopheles* spp. mosquito transmission (Johnson, 1988), has evolved independently from its sister CHIKV with divergence at least hundreds of years ago and probably in the much more distant past. Confounding factors such as purifying selection, which dominates the evolution of alphaviruses (Weaver et al., 2012; Weaver and Barrett, 2004), can render unreliable coalescent phylogenetic estimates of the most recent common ancestors represented by deep internal tree nodes (Wertheim et al., 2013; Wertheim and Kosakovsky Pond, 2011). The presence of RNA viral sequences (although not yet alphaviruses) within the genomes of vertebrates for millions of years Katzourakis and Gifford (2010), inconsistent with coalescent estimates of the age of various genera and families, is another indication of this limitation.

Based on the distributions and phylogenetic relationships among CHIKV and ONNV sequences and those of related alphaviruses, and most parsimonious reconstructions of their movement, both viruses are believed to have evolved in sub-Saharan Africa, but the exact region is ambiguous. The ancestor of these viruses was probably transmitted among primates and perhaps other vertebrates by *Aedes* mosquitoes, with ONNV later adapting (uniquely for an alphavirus) for transmission by *Anopheles* vectors. Because *A. aegypti* also evolved in sub-Saharan Africa, it may have been a CHIKV vector to humans in ancient times. The domestic form *A. aegypti aegypti* is believed to have evolved from an ancestral, arboreal, zoophilic form *A. aegypti formosus.* The latter subspecies currently encompasses both wild and domestic populations across Africa, while *A. aegypti aegypti* has a nearly worldwide tropical and subtropical distribution with high degrees of anthrophily in all locations (Brown et al., 2011). Human-to-human transmission of CHIKV probably began with the domestication of this mosquito, as human settlements accompanied by water storage developed in Africa.

6. Comparisons between CHIKV and other mosquito-borne arboviruses

Although the emergence and spread of CHIKV and other arboviruses cannot be predicted with certainty, comparisons with other arboviruses can reveal similarities and repeating patterns that offer some predictive insights. The histories of the four serotypes of the flavivirus dengue virus (DENV), which have circulated in the same regions using an identical urban transmission cycle, are the best examples. Like CHIKV, DENV is believed to have evolved from an enzootic, arboreal arbovirus transmitted by *Aedes* mosquitoes, but one that circulated in Southeast Asia (Wang et al., 2000; Vasilakis et al., 2011). However, probably due to the evolutionary advantage of multiple serotypes selected by immune enhancement, where one serotype benefits from another via increased viremia during secondary infections (Ferguson et al., 1999), DENV evolved within these enzootic foci into at least 4 serotypes, while CHIKV maintained very limited genetic and antigenic variation within the 2 enzootic African lineages. This could reflect the more recent evolution of CHIKV or its lack of potential for immune enhancement. DENV also established enzootic cycles in Africa following its introduction from Asia, while there is no evidence that CHIKV has followed the inverse pattern to become enzootic in Asia.

Although CHIKV is an important cause of human disease, it remains eclipsed by DENV, which causes an estimated 390 million human infections annually (Bhatt et al., 2013). This dramatic difference in incidence, even when herd immunity to CHIKV remains lower than that for DENV in many regions, may reflect a longer history of stable DENV circulation in human populations (several of the serotypes are estimated to have emerged from enzootic cycles in Asia much longer ago than the estimates for the Asian CHIK lineage, the earliest extant one known). The occurrence of four DENV serotypes and the ability of immune enhancement to augment their circulation probably also give an edge to DENV as a human pathogen. However, the ability of CHIKV to use *A. albopictus* as an efficient vector may allow this virus to spread to temperate regions not amenable to DENV transmission.

The other arbovirus with striking evolutionary and epidemiologic similarities to CHIKV is yellow fever virus (YFV), which also evolved in sub-Saharan Africa in similar, if not identical enzootic cycles involving NHPs and arboreal *Aedes* vectors (Bryant et al., 2007; Wang et al., 1996). Like CHIKV, YFV has limited antigenic and genetic diversity in Africa, with no evidence of sequential infections or immune enhancement (Beasley et al., 2015).

Enzootic strains of all three viruses (DENV, CHIKV, YFV) appear to be capable of exploiting *A. aegypti* as an efficient urban vector without any detectable adaptation. This facilitated the transport of these via sailing ships during past centuries, where they caused periodic outbreaks in port cities by transporting both the viruses and mosquito, probably with onboard circulation among sailors and passengers. However, unlike CHIKV and DENV, YFV is not known to have been introduced into Asia, with no obvious explanation. However, unlike DENV, it did establish an enzootic cycle in the neotropics after its introduction with the slave trade, and this cycle is the source of all recent human infections in South America, where extensive urban circulation has not been detected in decades (Bryant et al., 2007).

The reasons that the American enzootic YFV strains have not emerged recently could include cross-protective immunity from other New World flaviviruses, or a reduced ability of American enzootic strains, compared to the African strains that were transported in the past to the Americas, to use *A. aegypti* as an urban vector. Vaccination, available soon after the last major American epidemics, may also play a role in some regions. However, vaccine coverage remains far from complete both in enzootic regions of Africa and South America, while urban outbreaks continue only on the former continent.

The factors shared in common between CHIKV, DENV and YFV that predispose them to emerge into urban, human-*A. aegypti*-human epidemic cycles probably include: (1) their moderate infectivity for this vector, whose anthropophilic behavior, ecology and tendency to feed on multiple human hosts during a gonotrophic cycle (Fig. 5) (Harrington et al., 2014) is ideal for transmission of a human arboviral pathogen; (2) their ability to generate human viremia, probably because they are adapted to use primates as enzootic amplification hosts. Many other zoonotic arboviruses are also capable of using *A. aegypti* for laboratory transmission, including several that circulate in tropical regions endemic for dengue (Weaver and Reisen, 2010). However, only the three viruses described above, associated with primate enzootic hosts, have established stable urban cycles.

Studies of a wide variety of emerging viral pathogens show a strong association with the role of NHPs as enzootic hosts and the potential for emergence as human pathogens (Woolhouse and Gaunt, 2007), and the history of CHIKV, DENV and YFV certainly supports this concept. Others that also use NHP hosts and can infect *A. aegypti*, and which therefore appear to have urban emergence potential, include Mayaro and Zika viruses, both of which are increasingly recognized as important human pathogens with widespread distributions in the New and Old Worlds, respectively (Weaver and Reisen, 2010). Although phylogenetic studies suggest that Mayaro virus has evolved in the neotropics, it is not known whether the broader distribution of Zika virus in Africa and Asia reflects recent introductions or perhaps a longer evolutionary history.

However, other enzootic arboviruses not associated with NHPs certainly have the potential to spread and emerge as human pathogens, though probably not through direct human–mosquito–human transmission. West Nile virus, now the most important cause of arboviral encephalitis in the New World, is a prime example (Roehrig, 2013). Other arboviruses with African origins that may have similar potential include Rift Valley fever virus, which exhibits broad vertebrate and mosquito vector host ranges. Although the introduction of this virus to other continents could have severe impacts on human and domesticated animal health, limited or lack of use of avian hosts may reduce its mobility and explain its lack of historic spread far out of Africa (Weaver and Reisen, 2010).

7. Likely future pattern of Chikungunya in the New World

The scope and duration of the American CHIKF epidemics will likely be influenced initially by the complete lack of CHIKV herd immunity, combined with CHIKV's higher rate of apparent infection compared to DENV, suggesting even larger outbreaks than typically seen with DENV. This could result in more CHIKF than recent levels of dengue fever. For example, with CHIKV now reaching northern Mexico and its completely CHIKV-naïve population,

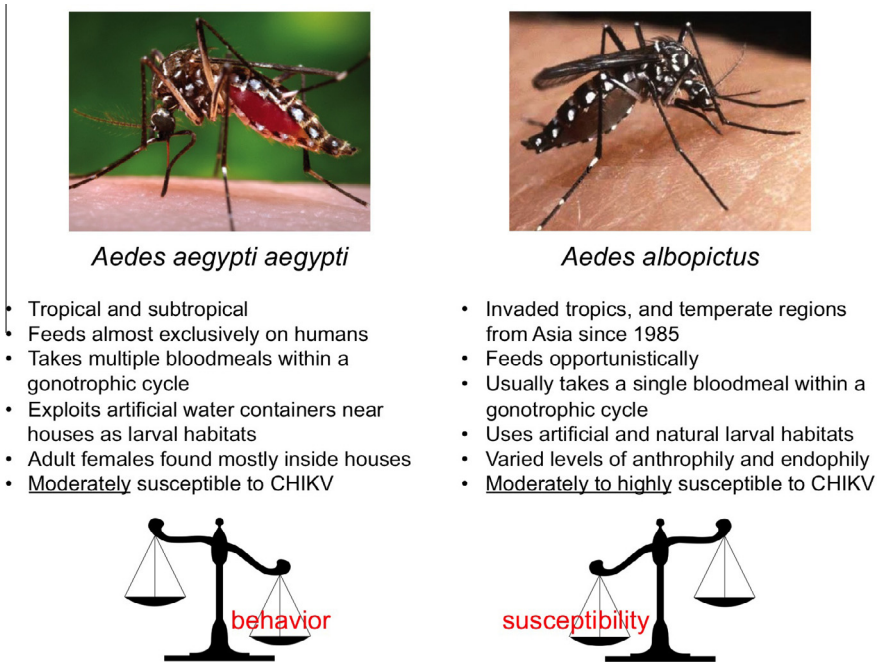

Fig. 5. Photographs of the two main urban mosquito vectors of CHIKV, *A. aegypti* and *A. aegypti*. Ecological and behavioral characteristics of each are listed below, and scales indicate that, when susceptibility is similar, the superior behavioral traits of *A. aegypti* result in its more important role as an urban CHIKV vector (as for dengue virus), while the increased susceptibility of *A. albopictus* to some CHIKV strains (Tsetsarkin et al., 2014) can compensate for its lesser anthrophily.

major epidemics near the U.S. border could result in a dramatic increase in imported cases due to the large number of persons who cross the border by land compared to air travelers, who have been exclusively importing CHIKV in recent years. With *A. aegypti* prevalent along most of the border, these imported cases represent an opportunity for CHIKV to initiate local circulation in the U.S. On the other hand, because CHIKV comprises a single serotype and there is no evidence of reinfection, dengue is likely to remain a major public health threat for a longer time period due to its four serotypes, resulting in multiple, repeated human infections (Gubler, 2011).

After initial outbreaks in the Western Hemisphere and the development of herd immunity, CHIKF is likely to decline in incidence, possibly followed by temporary regional extinctions, particularly on the smaller Caribbean Islands or other geographically isolated areas with limited human populations. The combination of accurate estimates of herd immunity and transmission modeling could prove highly valuable in predicting future transmission patterns. However, regardless of local extinctions, the propensity of CHIKV to spread efficiently via infected travelers indicates that it will retain the threat of periodic reintroductions and reemergence under favorable conditions. The history of *A. aegypti* transmission of the Asian CHIKV lineage since the 1950s, combined with our understanding of the epistatic constraints on its ability to adapt for enhanced *A. albopictus* transmission, suggest that initial epidemics in the Americas will mainly be restricted to the tropics and subtropics where the former vector thrives. However, an ECSA strain with the ability to adapt to *A. albopictus*, such as that reported recently in Brazil (Nunes et al., 2015) or an IOL strain that is already adapted, could be introduced at any time, which would place additional temperate and rural regions of the Americas, where this mosquito thrives, at higher risk.

If and when local CHIKV extinctions occur, and in transmission-permissive locations not yet affected, the rapid identification of imported cases will be critical to preventing the initiation of the human transmission cycle and potential spillback into an enzootic cycle (e.g. in Caribbean islands inhabited by African green monkeys). Especially for Asian lineage strains typically transmitted by *A. aegypti*, which has very limited dispersal potential, the rapid identification of an imported case can be accompanied by education of the patient and family members to minimize exposure to this vector, combined with vector control efforts in a 50–100 meter radius to kill mosquitoes that may already be infected. This approach is far simpler, more cost-effective and efficacious than attempting to control circulation after secondary cases and further spread have occurred. Critical to its success will be the local availability of diagnostics with turn-around times of a few days, the interval required to complete the extrinsic incubation period in the mosquito vector and permit subsequent transmission.

The ultimate stability of endemic CHIKF in the Americas seems likely, although there is no obvious explanation for its disappearance from the Indian subcontinent between 1973 and 2006. The stability of CHIKV in the Americas could also be determined by its ability to spill back into NHPs and potentially establish enzootic transmission foci, as YFV did several hundred years ago following its importation from Africa (Bryant et al., 2007). It is difficult to predict the likelihood of enzootic establishment, because there is no evidence that DENV has undergone the same scenario in the Americas, despite its presence for centuries (Carey, 1971), and because there are no data available on the amplification competence of New World NHPs for CHIKV, nor on the vector competence of sylvatic mosquitos from the neotropics.

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