

The Chikungunya threat: an ecological and evolutionary perspective

Christine Chevillon¹, Laurence Briant², François Renaud¹ and Christian Devaux²

¹ Génétique et Evolution des Maladies Infectieuses (UMR CNRS-IRD 2724), Centre IRD, 911 Avenue Agropolis, BP 64501 F-34095 Montpellier CEDEX 5, France

² Centre d'études d'agents Pathogènes et Biotechnologies pour la Santé (UMR CNRS-UMI-UMII 5236), Institut de Biologie, 4 Boulevard Henri IV, F-34905 Montpellier CEDEX 2, France

Chikungunya virus (CHIKV) is an emerging mosquito-borne alphavirus. Although primarily African and zoonotic, it is known chiefly for its non-African large urban outbreaks during which it is transmitted by the same vectors as those of Dengue viruses. Unlike Dengue viruses, CHIKV displays a re-emergence pattern that closely depends on long-distance migrations including recent re-immigrations from African (putatively zoonotic) sources. Genus-based differences also emerged when comparing the evolution of Dengue-related (*Flaviviruses*) and of CHIKV-related (*Alphaviruses*) arboviruses. In this review, we discuss current information on CHIKV genetics, ecology and human infection. Further investigations on African CHIKV ecology and the differences between *Flavivirus* and *Alphavirus* members in adaptive changes and evolutionary constraints are likely to help delineate the potential of further CHIKV (re-)emergence.

Chikungunya: an overlooked threat?

Chikungunya virus (CHIKV) was discovered in the mid-1950s from the serum of a febrile patient during a dengue-like epidemic in Tanzania, East Africa. The virus and associated disease were named from clinical symptoms: 'chikungunya' means 'stooped walk' in the local language, and refers to painful arthralgia that can last for months. Other clinical symptoms are similar to those of dengue fever, including fever, nausea, myalgia and rash. Since its discovery, CHIKV has been somewhat ignored by scientists except when it has caused large human outbreaks. The present review briefly summarizes our knowledge of the biology of alphaviruses and recalls the history of CHIKV emergence as seen by phylogenetic studies. The ecological determinants of CHIKV outbreaks are examined by combining the information gained on CHIKV genetic variations with current observations of CHIKV ecology and human infection reports. Finally we compare these results with the information gathered for more extensively studied emerging arthropod-borne viruses (arboviruses).

The main genetic and ecological traits of CHIKV

An alphavirus with arboviral ecology originating in West Africa

The genomic structure, replicative cycle and functions of viral loci and proteins have been extensively studied for different alphaviruses [1], providing predictive models for

CHIKV. **Box 1** details predictions about the CHIKV replicative cycle. Differences between vertebrate and invertebrate infections were repeatedly observed among alphaviruses [1] and are therefore expected to occur for CHIKV. At the level of the individual host, vertebrate immune systems usually clear infections within days, whereas invertebrates support lifelong infections. This has made it difficult to explain how human chikungunya disease could be characterized by long-lasting symptoms. A recent study might have provided the beginnings of an explanation by discovering CHIKV tropism for muscular satellite cells that can act as small reservoirs for virus or virus-encoded components (or both) for longer-than-expected periods [2]. At the cellular level, *Alphavirus* replication usually results in programmed host cell apoptosis in vertebrates – as recently confirmed for CHIKV in some human cell types [3] – but not in invertebrates. Finally, CHIKV replication requires viral proteins to interact with the host cell nucleus in vertebrate but not in invertebrate cells, but it is not known why. According to the predictive model depicted in **Box 1**, CHIKV and more generally *Alphavirus* genetic variations are expected ultimately to be constrained by a multiplicity of interactions; these can occur between viral components themselves, or between viral and host-encoded components, such as the recognition between NSP2 and viral polyprotein to produce the nonstructural proteins (NSPs), the association of NSPs into replication complexes, and the interaction of envelope complexes and host clathrin-coated vesicles to achieve viral entry in host cells.

At the viral level, alphaviruses experience a host-dependent balance between the mutation rate and evolutionary constraints, which entails the retention of more mutations when replicating in vertebrate cells than in invertebrate cells [1]. This has not yet been fully confirmed for CHIKV, but genomic variations linked to geographical origin and evolutionary rate have been studied using E1 or full-genome sequences [3–5]. Based on the strains isolated between the 1950s and the 1990s, the genetic variation of CHIKV is structured into three main clusters: the West African cluster, the Asian cluster and the Central, Southern and East African (CSEA) cluster, which includes genotypes circulating throughout Central, Southern and East Africa [3–5]. In contrast to the marked genetic homogeneity observed within the Asian cluster, a strong geographical pattern underlies the great genetic diversity occurring within the CSEA cluster [3–5]. The CHIKV viruses that emerged

Corresponding author: Chevillon, C. (christine.chevillon@mpl.ird.fr).

throughout the Indian Ocean area since 2000 demonstrated a limited sequence variation and were closely related to the sequence of a CSEA strain isolated 50 years earlier in Tanzania [5,6]. The addition of the CHIKV-related species O'nyong-nyong virus in phylogenetic studies resulted in the conclusion that the West African CHIKV cluster was ancestral, the CSEA cluster diverged from West African ancestors, and the Asian and Indian Ocean clusters of genotypes had lately and independently evolved from CSEA variants [3,5]. Therefore, CHIKV probably originated in West Africa before colonizing other African areas and emigrated twice from these newly colonized areas to invade Asia and then to circulate throughout the Indian Ocean. The pairwise analysis of genetic divergence between viruses that had been isolated seven years apart within the same area provided an average estimate of $6 \times 10^{-4} \pm 4 \times 10^{-4}$ changes per nucleotide per year within the E1 gene [3]. Such a calibration would date the appearance of CHIKV ancestors between 150 and 1350 years ago, that of the CSEA cluster between 100 and 800 years ago, and the emigration to Asia between 50 and 430 years ago [3] (Figure 1). This history has been marked by great qualitative changes in CHIKV ecology and human infection risk.

Human infection risk in Africa and complex ecology in native ecosystem(s)

With a few exceptions such as the 1969 Nigerian epidemics [7] and the Congolese epidemics in 2000 [4], CHIKV trans-

mission to humans in Africa has remained rural, with moderate impact on public health [8–10]. This suggests three nonexclusive hypotheses: (i) human CHIKV infections frequently are confused with other diseases; (ii) infection frequently is asymptomatic; and (iii) African human populations are regularly infected by zoonotic CHIKV and hence maintain a marked prevalence of anti-CHIKV immunity.

Disentangling the first two hypotheses would require developing CHIKV surveys among symptomatic patients and their healthy neighbors. Specific serological tools [11] and rapid reverse transcription-PCR-based tools [12] can help researchers improve these surveys even if public health services are likely to give priority to more life-threatening pathogens. Three studies incidentally showed elevated frequencies of human CHIKV infections in Africa, but none raised the question of CHIKV impact on public health. In Senegal (West Africa), a serological survey targeting diverse arboviruses reported seroconversion (hence contact with CHIKV antigens) in 35% of the people tested [13]. In Cameroon (central Africa), this frequency reached 45% in periods and areas where no CHIKV circulation was suspected and no medical alert had been raised [14]. In Malawi (southeast Africa), the search for risk factors for Burkitt's lymphoma syndrome incidentally revealed CHIKV seroconversion in 68% of surveyed patients and 50% of healthy controls [15].

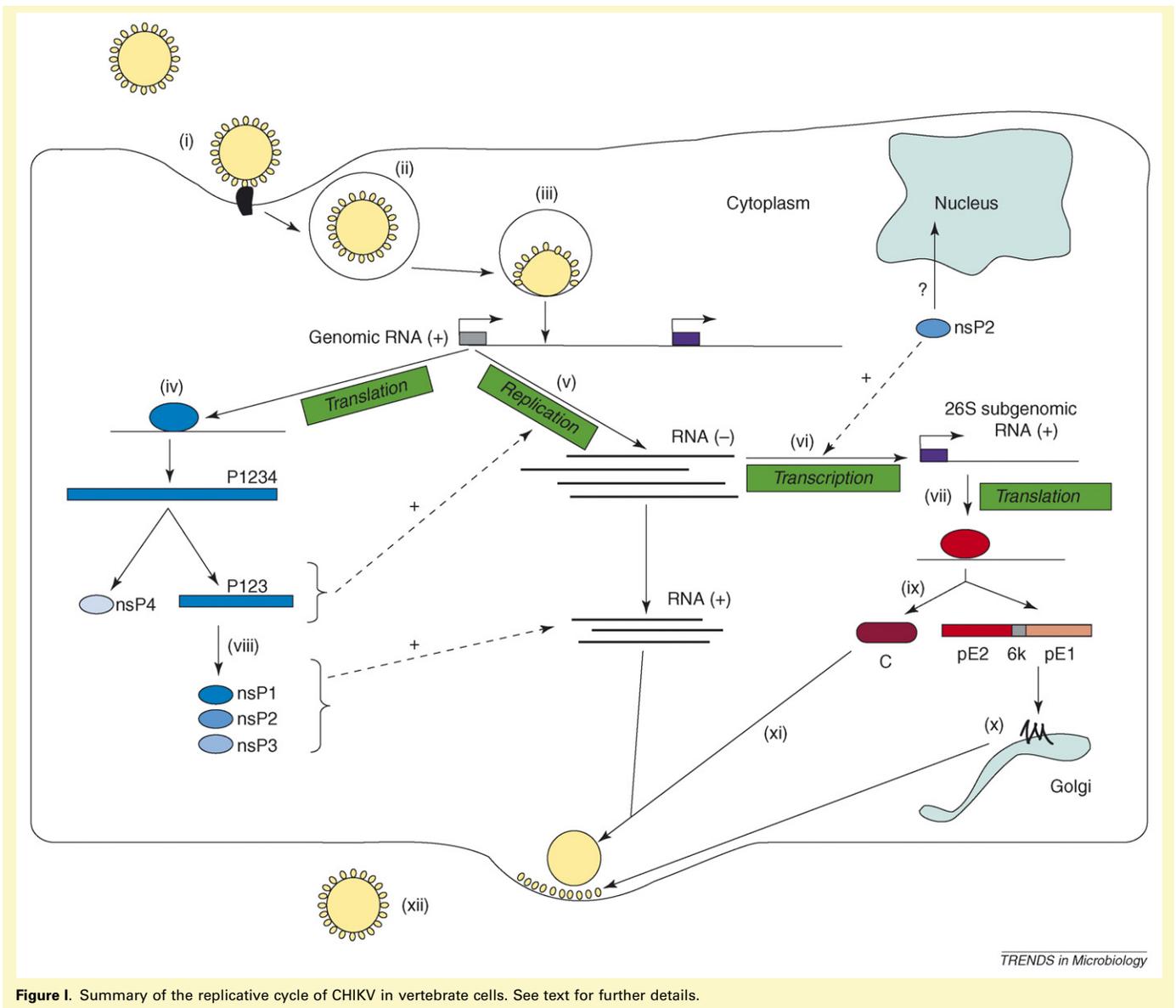
Box 1. The CHIKV genome and replicative cycle in vertebrate cells

How CHIKV loci determine the replicative cycle of the virus in vertebrate host cells can be predicted from our knowledge of other alphaviruses (see Refs [1,67,68] for reviews). Figure I focuses on the replication steps, which are numbered as in the text below. Figure II depicts viral loci and proteins, and the structure-function relationships in a genome common to members of the *Alphavirus* genus. The genome is a 5'-capped positive single-strand RNA, ~11.8 kb long, that harbors a poly(A) tail in its 3' end. It is composed of two open reading frames (ORFs) embedded between nontranslated regions (3' NTR and 5' NTR). The ORF located at the 5' end of the genome encodes a polyprotein precursor of nonstructural proteins (nsP1, nsP2, nsP3, nsP4) with replicative or proteolytic activities (or both). The second ORF encodes the polyprotein precursor of the structural proteins (C, E1, E2) forming the viral nucleocapsid and envelope.

CHIKV replication is likely to start with the attachment of the viral envelope to host receptors (step 1 in Figure I). Receptor(s) for CHIKV have yet to be identified, but a role has been proposed for the laminin receptor, glycosaminoglycans and DC-SIGN molecules in vertebrate cell infection by alphaviruses. The virus enters the target cell by endocytosis of clathrin-coated vesicles (step 2). During transfer to endosomes, the low pH environment leads to conformational reorganization of the E1-E2 viral envelope complex, exposure of the E1 fusion peptide and subsequent fusion with the endosomal membrane enabling delivery of the nucleocapsid into the cytoplasm (step 3). Then CHIKV replication proceeds along parallel pathways (steps 4, 5 and 6). Early in infection, the viral genome serves as an mRNA for the translation of the P1234 precursor and mature nonstructural proteins (step 4). RNA replication then occurs through the synthesis of a full-length minus-strand RNA intermediate (step 5) used as a template for synthesis of the genome-length RNA and for transcription of the 26S subgenomic plus-strand RNA from an internal promoter (step 6). Transcription of plus-strand RNA (produced at a constant rate throughout the replicative cycle) and minus-strand RNA (no longer detected at late infection stages) is temporally regulated by proteolytic processing of the P1234 nonstructural precursor. Early in infection, P1234 is cleaved into nsP4, which in association with

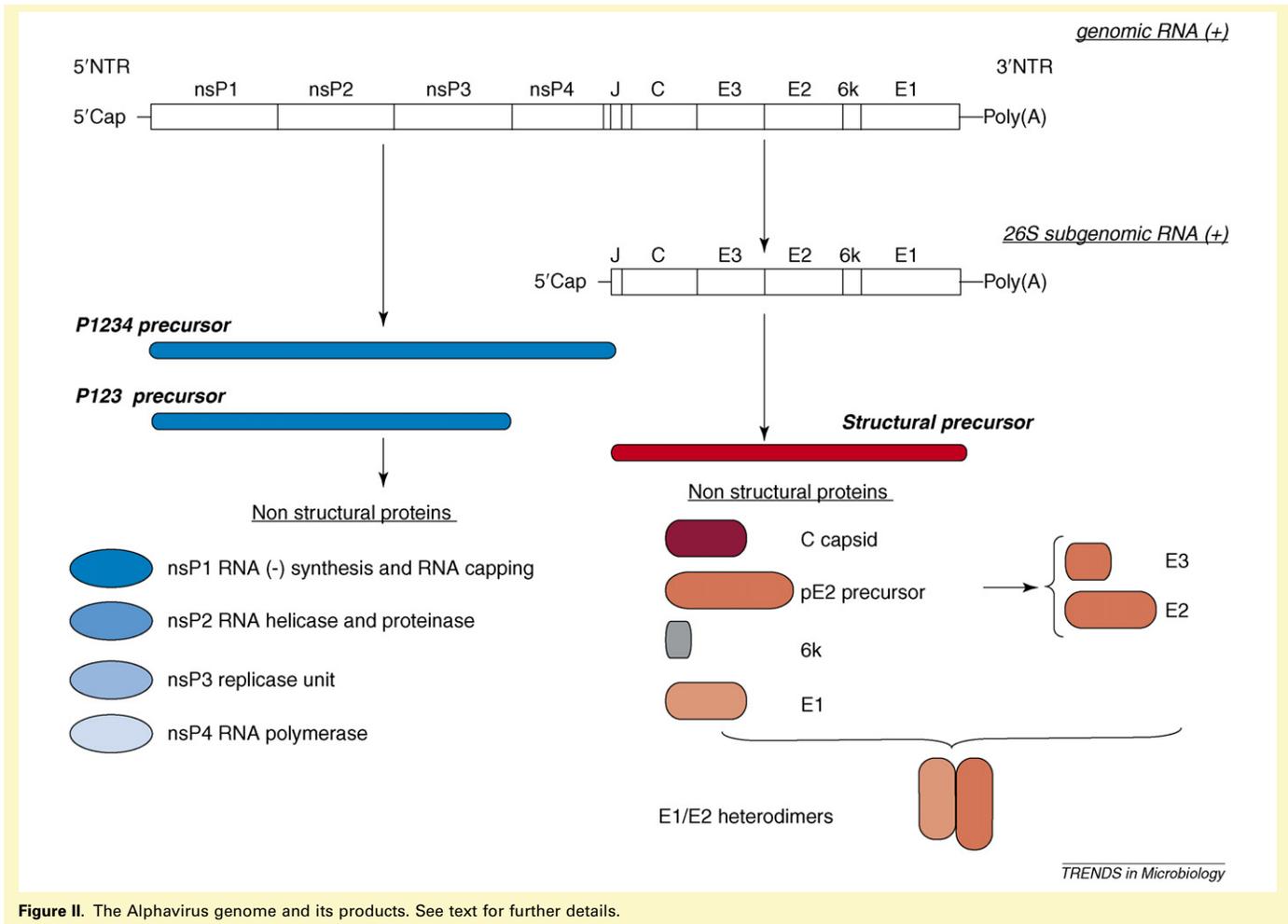
nsP123 polyprotein and host partners, probably behaves as a minus-strand RNA replicase. During replication, when the cell concentration of nsP123 is raised sufficiently, the precursor is further processed into mature proteins (step 8), which both regulate plus-strand RNA synthesis and amplify the subgenomic 26S positive-strand mRNA using the negative-strand RNA as a template (step 6). This step is regulated by helicase and proteinase functions of nsP2. A possible role was proposed for the migration of nsP2 into the nucleus of vertebrate cells, because *de novo* viral production is decreased in enucleated vertebrate cells.

In step 7, the 26S subgenomic RNA serves as mRNA for the translation of the C-pE2-6k-E1 polyprotein precursor and production of structural proteins. At late replicative steps the C-pE2-6k-E1 polyprotein precursor is processed cotranslationally and post-translationally into mature structural proteins. Autoproteolytic serine proteinase activity releases the capsid (C) from the N-terminus of nascent polypeptide (step 9). The envelope polyprotein precursor that inserts into the endoplasmic reticulum bilayer through an N-terminal signal sequence is processed into pE2 and E1. Step 10 then takes place in the Golgi complex, where E1 and pE2 associate and are exported to the plasma membrane. During transport, and probably just before arrival at the cell surface, pE2 is expected to be cleaved by furin or a related host proteinase into E2 and E3 as depicted in Figure II. Meanwhile, the C-terminus transmembrane insertion in endoplasmic reticulum bilayer is disrupted, thus enabling the release and maturation of the E1-E2 heterodimer in the viral envelope. This probably induces the reorientation of the E2 C-terminus from the luminal to the cytoplasmic side, required for the correct interaction between nucleocapsid and envelope glycoproteins. In parallel, mature nucleocapsids diffuse freely in cytoplasm toward the plasma membrane (step 11). Viral assembly is directed through electrostatic binding of the nucleocapsid to RNA and the recruitment of the plasma membrane-associated glycoproteins. The assembled alphavirus particle, which consists of an icosahedral nucleocapsid, finally buds through the cell membrane and becomes an enveloped virion (step 12).



Identifying an enzootic CHIKV transmission cycle would help determine whether these viruses tangentially spill over into human populations. Studies addressing this point have so far been biased by assuming that CHIKV vertebrate reservoirs were monkeys. CHIKV or anti-CHIKV neutralizing antibodies (or both) have been detected in monkeys [16,17] in addition to rodents [10], squirrels [10] and birds [10], with the vertebrate datasets remaining overall too anecdotal to be conclusive [17]. A 25-year survey conducted in Senegal provided more complete and slightly less biased information [10] by sampling CHIKV-infected mosquitoes in three ecosystems. Almost all sampling campaigns performed in the Sudan-Guinean forests around the village of Kédougou detected CHIKV-infected mosquitoes, with these mosquitoes representing all the simioanthrophilic species captured there (*Aedes furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus* and *Ae. neoaffricanus*). In the Sahelian village of Barkédji, none of the mosquito species captured, including *Ae. aegypti*, was CHIKV-infected. Finally, in the urban ecosystems located between Kédougou and Barkédji, CHIKV was

occasionally detected in *Ae. aegypti*. This pattern correlates with the detection of human cases of CHIKV infection in towns and in Kédougou, but not in Barkédji. Even if this study preferentially targeted the simioanthrophilic mosquitoes by using human baits for capture, it highlights differences in occurrence among ecosystems [10]. Interestingly, urban cases of human CHIKV infection or infection in *Ae. aegypti* consistently appeared after a pronounced peak in the number of CHIKV-infected mosquitoes in Kédougou [10]. Dispersal seemed to be related to the human migrations frequently observed between the Kédougou and urban ecosystems but not with Barkédji [10]. Overall, these data support: (i) enzootic CHIKV circulation in Kédougou-like ecosystems; (ii) recurrent viral spillover into the human populations living there; (iii) human-based emigration of CHIKV into towns where it can secondarily and temporarily be transmitted by *Ae. aegypti* mosquitoes; and (iv) the absence of a sustained urban cycle between humans and mosquitoes (*Ae. aegypti*) in the absence of recurrent viral immigration from rural Kédougou-like ecosystems.



In Kédougou, it was clearly established that CHIKV infection alternately peaked in two vector species, *Ae. furcifer* and *Ae. luteocephalus* [10]. Although confirmation is required by identifying their blood-feeding preferences, the competence for CHIKV and the frequency of acquired immunity against CHIKV in the vertebrate blood-meal source(s), the data could indicate that enzootic viruses regularly shift among different monkey species there. In addition to the original dataset of 12 074 samples (pools) of mosquitoes captured with human baits, the use of chicken baits enabled the capture of 77 pools of strictly ornithophilic mosquitoes, and that of sheep baits 12 394 pools of mosquitoes with variable blood-feeding behavior [10]. The published data are not sufficiently detailed to recompute a comparison of the minimal infection rates among mosquito species; nonetheless, it is noteworthy that CHIKV-positive pools were as frequent in strictly ornithophilic *Culex ethiopicus* than in the mosquitoes attracted by human baits (2 of 77 and 154 of 12 074 pools, respectively; Fisher exact test, $P = 0.26$). Moreover, 18 CHIKV strains were isolated from *Aedes* and *Anopheles* species that primarily blood-feed on sheep and cattle, secondarily on birds and unknown vertebrates, and occasionally on humans (see Table 3 in Ref. [10]) – that is, species that can serve as bridging vectors between transmission cycles. These data are not sufficient to establish definitively whether birds can act as efficient CHIKV reservoirs or if regularly infected domestic

animals further increase the risk of human infection within Kédougou-like ecosystems. However, they clearly indicate a more complex ecology than expected for West African CHIKV. Additional entomological surveys are urgently needed in other African rural areas where CSEA variants circulate. Indeed, the ecology of CSEA strains (i.e. identity and relative competence of vertebrate hosts and vectors) remains largely undocumented, with the exception of reports describing primate-to-*Ae. furcifer* transmission in South Africa [18] and occasional zebu infections in the Central African Republic [19].

CHIKV emergence in Asia: substantial impact on public health, simple ecology, and low sustainability in transmission

Human CHIKV epidemics have been documented in India [20,21], Thailand [22,23], Singapore [24], Japan [25], the Philippines [26,27], Indonesia [28] and Burma [29] throughout the 1960s and 1970s. These urban outbreaks had substantial impacts on public health. For instance, the percentage of CHIKV-infected individuals in epidemics was 31% in Bangkok, Thailand [22], and ranged from 15% to 25% in Vellore, India [21]. The rapid spread of invading CHIKV strains throughout Asia was indicated by the genetic similarity of the strains [3]. The primary vector was *Ae. aegypti*, a strictly urban and anthropophilic vector in this continent [29], and CHIKV epidemics in humans

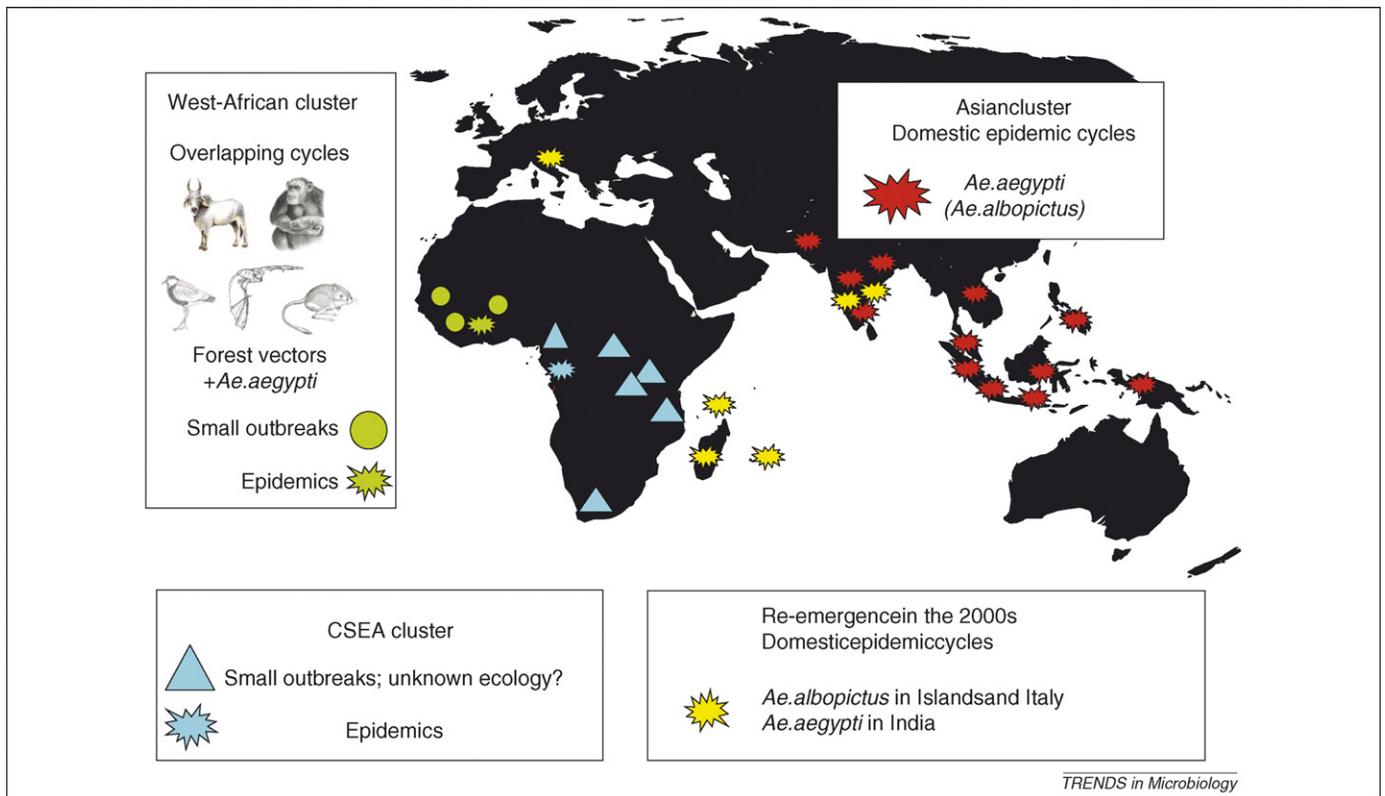


Figure 1. Historical and geographical variations in CHIKV ecology and associated risks for human health. Viral phylogenetic groups of genotypes are color coded: the ancestral West African group is in green; the second African cluster (or CSEA cluster, for Central, Southern, East African viruses) is in blue; the primarily Asian group is in red, and the CHIK viruses involved in Indian Ocean outbreaks since 2000 are represented in yellow. For each group, a box summarizes the knowledge acquired on CHIKV ecology and human infection risks (see details in text).

seemed to be disconnected from zoonotic transmission [30]. This conclusion has not been challenged in the rare cases where *Ae. albopictus*, a simioanthropophilic mosquito in Asia, was suspected as a secondary vector [23]. Overall, these epidemics most probably resulted from the immigration of CHIKV into urban ecosystems containing dense and naive human populations interspersed with a pronounced density of CHIKV-competent mosquitoes that principally blood-feed on humans.

An unexplained caveat concerns the long periods between epidemics in Asia. CHIKV re-emerged after a 20-year and a 32-year hiatus in Indonesia [28] and India [6,31], respectively. These periods were far too long to be explained only by a lack of CHIKV-naive humans (herd immunity) given the high human birth rates in those countries. Recent CHIKV re-emergence in India as a result of the immigration of an African variant [6] could provide a possible explanation, noting that this parallels the Senegalese patterns where urban transmission by *Ae. aegypti* lasts for only short periods of time after CHIKV (re-)immigration from forest ecosystems [10]. This suggests that, if globalization puts Asian cities at risk for large CHIKV epidemics, such risks would probably remain transitory on a local basis, and the human–*Ae. aegypti* cycle hopefully would lack sustainability at a local scale in the absence of continued CHIKV importation. In other words, the persistence of the Asian CHIKV genotype involved in epidemics of the 1960s–1990s is much more likely to have resulted from viral migration back and forth among cities and outbreaks than to an enhanced sustainability of the

human –*Ae. aegypti* cycle within any particular Asian locality.

Emergence in the Indian Ocean region: a remake of the Asian story?

The 2005–2006 emergence of CHIKV in the Indian Ocean area is reminiscent of the Asian configuration. The outbreaks had a significant impact on public health on the Indian Ocean islands at a time when eight Indian provinces also reported several thousand CHIKV infection cases [5,6,31,32]. All these epidemics involved genetically similar viruses suggesting that they were clearly related to a unique viral spreading event, probably originating from southeastern Africa [5,6]. Moreover, there was no evidence of zoonotic transmission on these islands, where CHIKV was found to be exclusively transmitted by *Ae. albopictus* [6], a species strictly anthropophilic there [33]. This pattern can be explained by the immigration of an infected carrier into places where CHIKV-competent anthropophilic vectors and naive human populations coexist at high densities.

Some mutations in the viral envelope had increased in frequency along the latest the Indian Ocean outbreak [5]. This phenomenon was interpreted as resulting from a selected adaptation to the local transmission by *Ae. albopictus* [5]; the first evidence in support of such a selection hypothesis was recently published [34]. It was also proposed that CHIKV has increased its virulence toward humans during this last emergence, during which the risk of death reached 0.5% and mother-to-child viral trans-

mission was documented [35]. This point requires further investigation because these outbreaks were among the few where patients benefited from intensive medical care, although fatal infections had previously been reported for Asian genotypes [36,37]. The 2005–2006 emergence was also associated with multiple CHIKV infections in travelers returning to their northern-hemisphere countries, where all the viruses isolated and sequenced were related to the CSEA cluster [38]. Although confirmation is still required through viral sequencing, such a globalization of human traffic looks also to be the most plausible explanation for the 170 cases observed in Italy during the summer of 2007 [39].

Characteristics of CHIKV as an emerging arbovirus

Our review has revealed five main characteristics of CHIKV epidemiology. First, CHIKV host diversity remains greater in its native ecosystem than in recently colonized areas. Second, the CHIKV impact on local public health is inversely related to its local ecological complexity. Third, human migration among non-African urban ecosystems and from African non-urban to urban Senegalese ecosystems seems to be the most likely mechanism responsible for CHIKV dispersal and epidemic emergence. Fourth, the colonization of urban ecosystems is associated with vector changes and the unsustainability of urban transmission at local scale; additional data are required to determine whether viral genetics or other extrinsic factors have shaped these modifications. Fifth, the two episodes of emigration out of Africa, in addition to the Tanzanian epidemics during which CHIKV was originally discovered, involved related viruses from the CSEA cluster. This point reinforces the need to investigate the ecology and the associated human-infection risks for non-urban African CHIKV belonging to the CSEA cluster. In particular, epidemiological investigations aimed at testing

the intensity of contact between humans and CHIKV zoonotic vectors in ecosystems where CSEA rather than West African genotypes circulate is needed to understand factors involved in epidemic emergence.

Host shifts as an ecological rule of arbovirus emergence

Changes in vectors or vertebrates (or both) are common characteristics of arbovirus emergence (see Refs [40–42] for reviews). These shifts are usually incomplete, so that epidemic crises require zoonotic viruses recurrently to spill over from animal host populations experiencing epidemics. This is also true for African yellow fever virus, which has invaded and become established in South America through its adaptation to new zoonotic cycles: human epidemics on both sides of the Atlantic remain dependent on occasional spillover from local zoonotic viruses [43]. The only exceptions are Dengue viruses (DENV) for which the native monkey-centered and the urban human-centered cycles have been disconnected for a long time [44,45]. The increase in transcontinental travel has facilitated the invasion of urban ecosystems not only by DENV, but also by their peridomestic mosquito vectors, *Ae. aegypti* and *Ae. albopictus* [46–48] (Figure 2). This led to a ‘snowball effect’ resulting in dengue pandemics; that is, the increase in the number of areas colonized by at least one vector species and one DENV strain directly multiplied their probability of invading new areas.

One can argue that CHIKV epidemiology fits either the general zoonotic or the DENV pattern depending on the geographical area considered. In Senegal, CHIKV epidemics in humans depend more or less directly on the spillover of zoonotic viruses. This might also be the case in other African regions. Long-range human migrations have so far connected the CHIKV epidemics observed among non-African urban ecosystems, raising the question of a risk for future CHIKV pandemics as previously

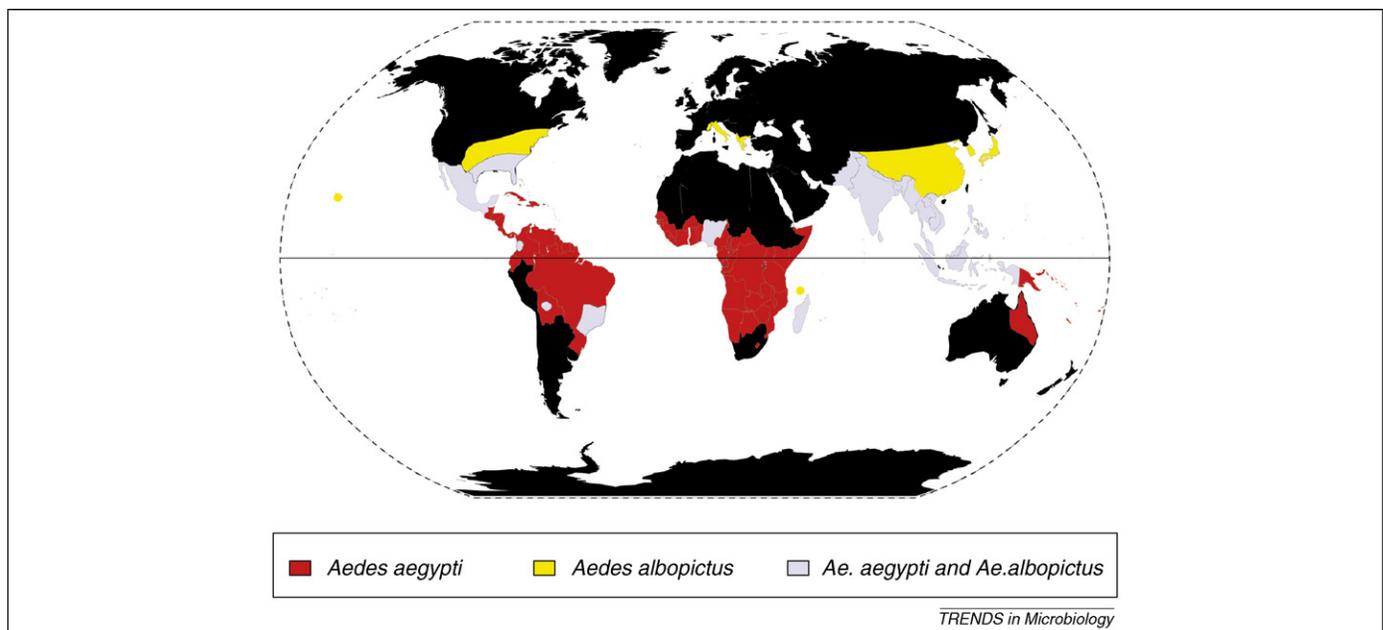


Figure 2. Current distribution of *Ae. aegypti* and *Ae. albopictus*. The *Ae. aegypti* genotypes that invaded the tropical belt belong to the anthropophilic subtype *Ae. aegypti aegypti*, and most probably originated from African Atlantic populations. Whereas the origins of the invasions of the Asian *Ae. albopictus* vector remain to be elucidated, the current active invasion by this vector of nontropical urban ecosystems in the New World and Mediterranean area should be noted.

observed for DENV. However, the low sustainability of CHIKV urban transmission at a local scale supported by long interepidemic periods has to be considered. Balancing the arguments for and against the likelihood of CHIKV pandemics requires addressing whether CHIKV and DENV share the same ability to complete their adaptation to new transmission cycles, that is, ultimately to sustain urban epidemics in the absence of any reimmigration of zoonotic-derived variants.

Emergence, host shifts and arbovirus genetics: contrasting patterns

DENV and CHIKV belong, respectively, to the *Flavivirus* and *Alphavirus* genera, which apparently differ in their genetic evolution with regard to host shifts. Among flaviviruses, adaptation to the vector(s) seems to be the major factor shaping both long- and short-term evolutionary processes. Ancestral *Flavivirus* divergence events match vector taxonomy: they discriminate the mosquito-borne, tick-borne and no-known-vector viruses independently of the taxonomy of vertebrate hosts [49–51]. Furthermore, viral base composition and codon usage by flaviviruses are usually specific for their carrier arthropods [52]. Also, the vertebrate pathologies correlate with vector taxonomy among mosquito-borne flaviviruses: *Culex*-transmitted viruses tend to promote neural disorders, whereas *Aedes*-transmitted viruses are more likely to induce hemorrhagic pathologies [51]. In addition, the divergence among the tick-borne flaviviruses differentiates viruses that infect continental rodents from those infecting seabirds (i.e. vertebrates likely to be colonized by different ticks). Overall, this would argue for a vertebrate–flavivirus interaction having evolved chiefly as a by-product of the flavivirus adaptation to the vector. This apparent predominance of the vector-driven evolution of flaviviruses into a vertebrate-driven evolution has apparently remained pivotal in recent periods. Dengue is a spectacular example. If human DENV can infect several *Aedes* species, the emergence of DENV is considerably accelerated once *Ae. aegypti* and *Ae. albopictus* invade urban ecosystems. In other words, even if we cannot exclude the possibility of DENV adaptation to humans (but see counter-evidence in Ref. [53]), the pandemic mechanism is already fully explained by viral specializations for a few invading mosquito species within a narrow but ubiquitous niche (i.e. tropical urban ecosystems). Similarly, the introduction of West Nile virus in the New World resulted in the infection of several dozen new species (mosquitoes and birds) [54,55] and has been marked by a modified but selected virulence pattern (mediated by thermoresistance enabling some viruses to keep replicating in some febrile birds) [56,57]; but its epidemic dynamics remain mostly constrained in the New World, as in the Old World, by the ancestral vector [58].

A different balance between vector-dependent and vertebrate-dependent evolution is observed among alphaviruses. Their ancestral divergences correlate neither with the vector nor with vertebrate taxonomy [59,60]. Pathologies in vertebrate hosts correlate only with the geographical origin of the virus: New World alphaviruses tend to cause encephalitis, whereas Old World alphaviruses tend

to cause rash, arthralgia and arthritis [61,62]. This pattern is not easily explained by *Alphavirus* evolutionary divergence, because the pattern arose despite transatlantic viral migrations [60]. Moreover, the vector-dependent and vertebrate-dependent selection pressures seem more independent when acting on alphaviruses than on flaviviruses. For example, the O'nyong-nyong virus diverged from CHIKV by acquiring the adaptation to anopheline rather than culicine vectors [61] and by losing the aptitude to replicate in *Ae. aegypti* cells [62]. These changes have been correlated with restricted mutation in the 3' noncoding end of the viral genome [62] but did not result in modifications of clinical symptoms [61]. Furthermore, our knowledge about Venezuelan equine encephalomyelitis virus (VEEV) has raised questions regarding the persistence of the mutations enabling adaptation to new hosts and vectors; for VEEV this involved the genetic adaptation to an amplification cycle – defined by horses and *Aedes (Ochlerotatus) taeniorynchus* – required to bridge the zoonotic cycle – defined by rodents and *Culex* mosquitoes – subsequently to emerge in human populations [63]. Indeed, each spillover event was found to require the acquisition by independent VEEV strains of a particular mutation in the E2 gene sequence enabling increased viremia in infected horses to facilitate transmission by *O. taeniorynchus* [64,65]. However, only a restricted number of horse-adapted strains are capable of infecting *O. taeniorynchus*. Moreover, this recurrent mutation pattern is not retained in long-term VEEV evolution but needs to be reacquired and reselected whenever the ecological situation favors a new cascade of host-shift events [63–65].

Overall, adaptation to a new vector seems to be the most pivotal step of flavivirus emergence, but this is just one of several disconnected but necessary steps of alphavirus emergence. If so, flaviviruses should more easily achieve pandemics than alphaviruses when adapting to vectors as widely distributed as *Ae. aegypti* and *Ae. albopictus*, unless cross-immunity among flaviviruses prevents the diffusion of a second emerging flavivirus; this is one way DENV immunity is thought to have prevented yellow fever pandemics [43]. More data are required to settle the robustness of this hypothetical flavivirus–alphavirus contrast regarding the balance between invertebrate- and vertebrate-based evolutionary constraints. Moreover, it would be worth investigating the possible molecular bases of such a contrast; would it result from stronger epistasis in the flavivirus genome, with its single open-reading frame (ORF), than in the alphavirus genome with its two ORFs? Alternatively, could it result from a different diversity in the host components interacting with viral components across the vertebrate and invertebrate infection process? Addressing such questions of fundamental virology would provide crucial information necessary to understand and forecast the emergence of new epidemics.

Consequences regarding the CHIKV pandemic risks

The fear of CHIKV pandemics arose on the strength of evidence that CHIKV accumulates the ability for rapid long-distance migration and the possibility of transmission through competent urban vectors (*Ae. aegypti* and *Ae. albopictus*) – two ecological traits of DENV. However, in

urban ecosystems where DENV are endemically transmitted, CHIKV outbreaks remain separated by long inter-epidemic periods unless reimmigration occurs, indicating that something is preventing the complete establishment of CHIKV, that is, a complete independence of human-centered transmission from zoonotic transmission. Possible explanations can be derived from accumulating data on variation in patterns of vectorial capacity for both DENV and CHIKV within the invading vectors *Ae. aegypti* and *Ae. albopictus*. Investigating further the potential for an *Alphavirus-Flavivirus* evolutionary contrast is also likely to be informative. If CHIKV re-emergence better fits the VEEV than the DENV models, then the risk for a CHIKV pandemic would be reduced: African zoonotic CHIKV would each time have to acquire the mutations able to maintain pronounced viremia in putative intermediate vertebrate hosts before ultimately achieving re-emergence in human ecosystems. Testing this latter hypothesis requires identifying the host-shift events occurring in Africa for the CHIKV belonging to CSEA and West African clusters when immigrating into urban ecosystems; comparing the mutations that enable vector infection with those affecting vertebrate viremia; and tracking the fate of such mutations along the long-distance migration connecting re-emerging epidemics. Noting that CHIKV punctually re-emerges in DENV endemic areas, extending the studies on *Alphavirus* exclusions [66] to *Flavivirus-Alphavirus* superinfection patterns would further help understanding the global pattern of CHIKV (re-)emergence.

Concluding remarks and future perspectives

This overview has raised questions regarding the mechanisms that might enable forecasting of CHIKV (re-)emergence (Box 2). It has also raised issues that should be considered when investigating pathogen emergence, especially the pivotal importance of the ecology and diversity of the emerging pathogen in its geographical and historical settings. For CHIKV, urban epidemics might represent only the tip of the iceberg, and the primary source of urban re-emergence could lie in African rural ecosystems. Identifying CHIKV zoonotic transmission in African rural ecosystems and avoiding the sampling biases of previous studies are therefore urgent priorities. Research is also needed to establish how these pulses of change and amplification are achieved, through quantification of human migration into and from these rural ecosystems and identification of the potential amplification cycles occurring at the border of such ecosystems. In addition, after DENV, SARS and avian flu virus, the CHIKV example has reinforced the notion that human activities are the main causes of viral emergence. For CHIKV, humans have mediated the long-distance migration of viruses into and among urban ecosystems, have facilitated the settlement of competent vectors in urban ecosystems, and could even create some African amplification cycles through farming activities near sylvan ecotones. Finally, questions regarding the balance of vertebrate-based and invertebrate-based evolutionary constraints are raised by the *Alphavirus-Flavivirus* comparison. Overall, the CHIKV case illustrates well how questions in virology, vector biology, ecology, genetics

Box 2. Issues to address for better forecasting of CHIKV epidemics

- Is the Senegal case representative of all African zoonotic transmissions?
- Are there genetic mutations responsible for triggering the ecological changes of CHIKV from forest to human-made ecosystems in Africa?
- What are the specific features of CHIKV ecology and epidemiology in ecosystems where zoonotic CHIKV belong to the CSEA cluster? Do they explain why the zoonotic source of epidemics tends to display CSEA genotypes rather than West African ones?
- Is variation in CHIKV virulence to humans the same within native and epidemic areas?
- Are the CHIKV mutations that were associated with transmission by *Ae. albopictus* in the Indian Ocean islands [5] also found in Asian areas where this vector species was suspected to act as a secondary vector [23]?
- Outside Africa, how does CHIKV interact with other endemic or emerging pathogens? In particular, how do CHIKV and DENV interact when and where they cocirculate within the same transmission cycles?
- More generally, how much does the balance between invertebrate-induced and vertebrate-induced evolutionary constraints actually differ between flaviviruses and alphaviruses? Does the apparent contrast evident from available data result from sampling biases in either genus?
- When and where will CHIKV emerge in North America where urban vectors are also present? How long will the Italian or putative North American epidemics persist without reimmigration?

and human behavior provide complementary pieces in the puzzle of arbovirus emergence.

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