Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography

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Phylogenetic analysis of the Flavivirus genus, using either partial sequences of the non-structural 5 gene or the structural envelope gene, revealed an extensive series of clades defined by their epidemiology and disease associations. These phylogenies identified mosquito-borne, tick-borne and no-known-vector (NKV) virus clades, which could be further subdivided into clades defined by their principal vertebrate host. The mosquito-borne flaviviruses revealed two distinct epidemiological groups: (i) the neurotropic viruses, often associated with encephalitic disease in humans or livestock, correlated with the Culex species vector and bird reservoirs and (ii) the non-neurotropic viruses, associated with haemorrhagic disease in humans, correlated with the Aedes species vector and primate hosts. Thus, the tree topology describing the virus–host association may reflect differences in the feeding behaviour between Aedes and Culex mosquitoes. The tick-borne viruses also formed two distinct groups: one group associated with seabirds and the other, the tick-borne encephalitis complex viruses, associated primarily with rodents. The NKV flaviviruses formed three distinct groups: one group, which was closely related to the mosquito-borne viruses, associated with bats; a second group, which was more genetically distant, also associated with bats; and a third group associated with rodents. Each epidemiological group within the phylogenies revealed distinct geographical clusters in either the Old World or the New World, which for mosquito-borne viruses may reflect an Old World origin. The correlation between epidemiology, disease correlation and biogeography begins to define the complex evolutionary relationships between the virus, vector, vertebrate host and ecological niche.

Introduction

The Flavivirus genus contains many viruses associated with emerging and re-emerging human diseases, including dengue haemorrhagic fever, Kyasanur Forest haemorrhagic disease, Japanese encephalitic disease, Rocio virus encephalitis (Monath & Heinz, 1990) and West Nile fever (Lanciotti et al., 1999).

Elucidating the evolution of viruses is particularly valuable for understanding the origin and spread of emerging and re-emerging diseases (Holmes, 1998).

Flaviviruses are a useful model for studying the evolution of vector-borne virus diseases, since they comprise mosquito-borne, tick-borne and no-known-vector (NKV) viruses (Porterfield, 1980). The genus contains about 70 recognized flaviviruses that are antigenically related and have a widespread geographical dispersion. They are positive-stranded RNA viruses with a genome of approximately 10.5 kb. Virions contain three structural proteins, capsid (C), membrane (M) and
Table 1. Flaviviruses analysed in this study

All of the flaviviruses that were analysed in this study are classified by their virus group (Heinz et al., 2001).

<table>
<thead>
<tr>
<th>Virus group</th>
<th>Flavivirus analysed, excluding flavivirus E gene sequences (presented separately)</th>
<th>Abbreviation</th>
<th>Flavivirus partial E genes sequenced in this study</th>
<th>Abbreviation</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louping ill</td>
<td>Louping ill Spanish sheep encephalitis Turkish sheep encephalitis Greek goat encephalitis</td>
<td>LI SSE TSE GGE</td>
<td>Gadgets Gulley</td>
<td>GGY</td>
<td>AF372408</td>
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<tr>
<td>Tick-borne encephalitis</td>
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<td>FETBE WTBE</td>
<td>Kadam</td>
<td>KAD</td>
<td>AF372420</td>
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<tr>
<td>Mammalian tick-borne</td>
<td>Kyasanur Forest disease Langat Omsk haemorrhagic fever Powassan Royal Farm Karshi</td>
<td>KFD LGT OHF POW RF KSI</td>
<td>Meaban</td>
<td>MEA</td>
<td>AF372423</td>
</tr>
<tr>
<td>Seabird tick-borne</td>
<td>Tyuleniy Saumarez Reef</td>
<td>TYU SRE</td>
<td>Aroa</td>
<td>AROA</td>
<td>AF372413</td>
</tr>
<tr>
<td>Aroa</td>
<td>Iguape</td>
<td>IGU</td>
<td>Bussuquara</td>
<td>BSQ</td>
<td>AF372410</td>
</tr>
<tr>
<td>Dengue</td>
<td>Dengue types 1–3, 4 Kedougou</td>
<td>DEN 1–3, 4 KED</td>
<td>Alfuy</td>
<td>ALF</td>
<td>AF372406</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>Japanese encephalitis Murray Valley encephalitis Saint Louis encephalitis Usutu Kunjin Yaounde</td>
<td>JE MVE SLE USU KUN YAO</td>
<td>West Nile (tick)</td>
<td>WN (tick)</td>
<td>AF372405</td>
</tr>
<tr>
<td>Ntaya</td>
<td>Tembusu THCr*</td>
<td>TMU THCr*</td>
<td>Bagaza</td>
<td>BAG</td>
<td>AF372407</td>
</tr>
<tr>
<td>Spondweni</td>
<td>Banzi Boubouï Potiskum Sepik Uganda S Yellow Fever</td>
<td>BAN BOU POT SEP UGS YF</td>
<td>Edge Hill</td>
<td>EH</td>
<td>AF372419</td>
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<td>Yellow Fever</td>
<td>Kokobera Stratford Entebbe bat Sokoluk Yokose</td>
<td>KOK STR ENT SOK YOK</td>
<td>Saboya</td>
<td>SAB</td>
<td>AF372421</td>
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<tr>
<td>Modoc</td>
<td>Apoi Cowbone Ridge Jutiapa Modoc Sal Vieja San Perlita</td>
<td>APOI CR JUT MOD SV SP</td>
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</tbody>
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envelope (E), and infected cells have been shown to contain seven non-structural (NS) proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Rice et al., 1985; Rice, 1996).

The evolution, dispersal patterns and epidemiological characteristics of flaviviruses are believed to have been determined through a combination of constraints imposed by the arthropod vector, the vertebrate hosts, the associated ecology and the influence of human commercial activity. For example, the clinal evolution of the tick-borne encephalitis (TBE) complex viruses across the Euro-Asian land mass reflects the life-cycle and feeding habits of the ixodid tick (Zanotto et al., 1999) combined with the appropriate rodent host species and climatic conditions (Randolph et al., 2000). Similarly, the introduction of goats and sheep onto the hillsides of Turkey, Greece, Spain, Ireland, Norway and the British Isles was followed by the appearance of louping ill virus (Reid, 1984; Gao et al., 1993; Gaunt et al., 1997; McGuire et al., 1998). The emergence and expansion of dengue haemorrhagic fever in the tropics has followed an increase in human and mosquito population densities brought about by urbanization and industrialization (Zanotto et al., 1996). Finally, the trans-Atlantic dispersal of yellow fever (YF) virus, and possibly many other flaviviruses, is thought to have coincided with the transportation of people and mosquitoes from Africa to the Americas on slave ships during the past few centuries (Strode, 1951; Gould et al., 1997).

Using detailed molecular phylogenetic analyses, we have attempted to bring all these factors together in order to understand the nature of flavivirus evolution, epidemiology and dispersal. In this study, maximum likelihood (ML) phylogenetic analyses of virtually all of the recognized flaviviruses were performed using partial NS5 gene sequences and the tree was compared with one based on the E gene sequences. The epidemiological and aetiological characteristics of each flavivirus have been mapped onto the phylogeny to reveal a striking pattern of coincidence between the topological arrangement of the viruses and their associated epidemiological characteristics.

### Methods

The flavivirus cDNA sequences determined in this study and the flavivirus abbreviations used hereafter are described in Table 1. Viral RNA extraction and RT–PCR procedures have been described previously (Gritsun & Gould, 1995; Gaunt et al., 1997). A nested set of primers encompassing the species-specific amino acid motifs defined previously (Marin et al., 1995) was designed to amplify a partial E gene locus for the genus flavivirus. The PCR primers amplified 85% of the flaviviruses described by Calisher et al. (1989) and will be described elsewhere. Amplified cDNA was cloned into pGEM T vectors (Promega) and sequenced using the Thermo-Sequenase Cycle Sequencing kit (Amersham), according to the manufacturer’s instructions. cDNA sequences were determined from two recombinant plasmids prepared from each virus and any differences were resolved using a third clone.

Phylogenetic analyses were performed using the partial NS5 sequences described previously by Kuno et al. (1998), with the addition of LI virus, strain 369 (Y07863). The flavivirus E gene sequences were obtained in this study and also included E gene sequences from previous studies (Marin et al., 1995; Billoir et al., 2000). Partial NS5 gene nucleotide sequences were aligned using CLUSTAL X and edited manually from their amino acid alignment (Thompson et al., 1994). The partial E gene sequences were exclusively aligned using their amino acid sequences and the final alignment comprised 308 amino acids (including gaps).

The robustness of the data sets was examined for deviations in nucleotide or amino acid base composition between taxa, as nucleotide or amino acid base homogeneity is a prerequisite for the mutation models used in this study. The NS5 nucleotide alignment required the removal of the third codon position to obtain nucleotide base homogeneity ($P^2$-test, $P < 0.05$ for all three codon positions and $P > 0.99$ for codon positions one and two only) (PAUP*, version 4.0; Swofford, 1999). The E gene amino acid alignment required the removal of cell fusing agent virus, which had sometimes been used in previous analyses, to obtain amino acid homogeneity under the same test ($P > 0.05$ for all other species) (Puzzle; Strimmer & von Haeseler, 1996). Nucleotide variation was examined using a sliding window analysis (SWAN; Proutski & Holmes, 1998). The NS5 gene was further investigated for possible saturation

### Table 1 (cont.)

<table>
<thead>
<tr>
<th>Virus group</th>
<th>Flavivirus analysed, excluding flavivirus E gene sequences (presented separately)</th>
<th>Abbreviation</th>
<th>Flavivirus partial E genes sequenced in this study</th>
<th>Abbreviation</th>
<th>Accession no.</th>
</tr>
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<tbody>
<tr>
<td>Rio Bravo</td>
<td>Rio Bravo</td>
<td>RB</td>
<td></td>
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<td>Bukalasa</td>
<td>BUK</td>
<td></td>
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<td></td>
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<tr>
<td>Carey Island</td>
<td>CI</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Dakar Bat</td>
<td>DK</td>
<td></td>
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<td>Montana myotis leukoencephalitis</td>
<td>MML</td>
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<td></td>
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<td>Phnom Penh</td>
<td>PPB</td>
<td></td>
<td></td>
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<tr>
<td>Batu Cave</td>
<td>BC</td>
<td></td>
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</table>

* Additional flavivirus analysed by Kuno et al. (1998).
using a 10 bp sliding window analysis, which estimated as an entropy function of the nucleotide variation (Var). The sliding window analysis of the mosquito-borne flaviviruses, and each mosquito-borne flavivirus clade described later, identifies a region between nucleotides 342 and 392 of the 693 bp NS5 gene alignment showing the highest level of variation (Var = 0.72–1.21). This region coincided a large alignment gap and proved difficult to align using amino acids; therefore, these nucleotides were subsequently removed. The ML model for NS5 gene nucleotide substitution was determined by testing 40 models of nucleotide substitutions (MODELTREE; Posada & Krandall, 1998), which described an eight parameter model consisting of the general time reversible (GTR) model of nucleotide substitution (six parameters), an invariant rate parameter (PINVAR) and the alpha parameter of a four category discrete gamma distribution (Γ; one parameter). The ML model parameters were estimated by an automated reiterative ML heuristic search (PAUP*) and from Jukes–Cantor distances (MODELTREE). Parameter estimates were incorporated into a full heuristic ML search for ten replications. The level of phylogenetic support was determined by bootstrap re-sampling using ML distances incorporating each parameter estimate and a full heuristic search for 1000 replications (PAUP*). In addition, 100 Monte Carlo DNA sequence simulations were performed from the final NS5 gene tree topology and reconstructed using the ML parameter estimates, as described previously (Seq-Gen; Rambaut & Grassly, 1997).

Evolutionary reconstructions of partial E gene amino acid alignments were performed by ML quartet puzzling using the JTT substitution matrix (Jones et al., 1992) for 10000 quartet puzzling steps (Puzzle; Strimmer & von Haeseler, 1996). Likelihoods were analysed for 0, 4, 8 and 16 discrete Γ categories and the presence or absence of an PINVAR was assessed using a likelihood ratio test.

The phylogenies obtained from the partial NS5 and E gene sequences of each flavivirus were tested for their association with particular arthropod vectors, disease associations (haemorrhagic and encephalitic) and their geographical distribution using the International Catalogue of Arboviruses (Karabatsos, 1995).

**Results**

**Phylogenetic reconstruction**

All NS5 gene phylogenies produced a single likelihood regardless of the taxa order in each full heuristic search performed (n ≥ 10 in all cases; Table 2). Specific details for building the ML NS5 gene phylogeny and the model parameters used are described in Table 2.

The robustness (convergence) of the E gene ML phylogeny was observed after 10000 quartet puzzling steps from two key indicators. Firstly, only 3% of the quartets for all of these searches was unresolved. Second, although six models of rate substitution were tested (see Methods), the quartet puzzling support differed by a maximum of 1%. Thus, no difference in branching order between the E gene models was observed, regardless of the combination of rate parameters used. The phylogeny for the significantly highest likelihood, as determined by likelihood ratio tests, was an eight category Γ distribution with PINVAR.

Figs 1 and 2 present the phylogenies for the NS5 gene and the E gene, respectively. The topologies showed congruence at all levels where bootstrap or quartet puzzling support was

<table>
<thead>
<tr>
<th>Nucleotide region removed</th>
<th>GTR</th>
<th>Calculation</th>
<th>No. full searches including PINVAR parameters</th>
<th>Likelihood (ln)</th>
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<td>1. L-C</td>
<td>2.536</td>
<td>1.804</td>
<td>1.005</td>
</tr>
<tr>
<td>None</td>
<td>2. L-M</td>
<td>1.604</td>
<td>1.002</td>
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<tr>
<td>Hyper-variable</td>
<td>3. L-C</td>
<td>1.541</td>
<td>1.001</td>
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<tr>
<td>Hyper-variable</td>
<td>4. L-M</td>
<td>1.541</td>
<td>1.001</td>
<td></td>
</tr>
</tbody>
</table>

A full heuristic ML search incorporating the parameter estimates.
greater than 60%. Equivocal incongruence was observed for SLE, AROA, BSQ and NJL virus monophyly and the DEN viruses between the two phylogenies presented in Figs 1 and 2.

As demonstrated previously (Kuno et al., 1998), the NS5 gene phylogeny defined three major groups comprising the mosquito-borne, tick-borne and NKV flaviviruses. However, both analyses showed that the three NKV bat-associated viruses, ENT, SOK and YOK viruses, were grouped within the mosquito-borne virus clades forming a basal lineage with YF and SEP viruses.

**Aedes clades and the Culex monophyly**

Mapping epidemiological and disease characteristics of the individual mosquito-borne viruses onto the phylogenetic trees revealed a correlation between the principal vector genera (Culex and Aedes species), the principal vertebrate hosts (birds and/or mammals) and the virus tropisms in humans and livestock (neurotropic versus non-neurotropic) (Figs 1 and 2). The mosquito-borne viruses could be divided into two epidemiologically distinct vector groups, those that were primarily isolated from Aedes species and those that were
Fig. 2. Phylogeny tree of the genus *Flavivirus* based on the E gene amino acid sequence constructed using the JTT substitution model and incorporating an additional two parameters ($\Gamma$ and PINVAR). Nomenclature is identical to that in Fig. 1.

primarily isolated from *Culex* species. The 17 flaviviruses that were primarily isolated from *Aedes* species formed two paraphyletic groups, one containing YF virus and the other containing the DEN viruses. In these two paraphyletic groups, 82% of mosquito-borne flaviviruses are known to be associated with *Aedes* species (14/17), hereafter denoted as the *Aedes* clades. The other viruses in these clades, i.e. SEP, POT, BAN and SAB, have been primarily associated with *Mansonia* species, rodents, *Culex* species and sandflies, respectively. The major exception is the mosquito species *Haemagogus*, the sylvatic vector of YF virus in South America, although this does not apply to the urban vector. The flaviviruses primarily isolated from *Culex* species formed a single clade of 23 viruses, which included JE, SLE and WN viruses as notable examples. The mosquito vector was identified for at least 16 of these viruses (Karabatsos, 1995). Of these 16, 89% of mosquito-borne flaviviruses in the *Culex* species monophyly are known to be isolated from *Culex* species (14/16), hereafter denoted as the *Culex* clade.

Figs 1 and 2 show that there is a clear correlation between the virus, mosquito vector species and associated host. Both *Aedes* clades contained viruses that were maintained in sylvatic primate cycles, namely YF, DEN and ZIK viruses, or other mammals, while birds were not strongly associated with any of the viruses in the *Aedes* clades. Although *Aedes* clade viruses may infect birds, they are believed to be ‘dead-end hosts’. In contrast, none of the *Culex* clade viruses are maintained in primate cycles. Moreover, a high proportion of flaviviruses in the *Culex* clade are associated with mosquito–bird cycles (at least 12/23 viruses) (Figs 1 and 2). However, mammals are additionally involved in the persistence of *Culex* clade viruses, although many are considered dead-end hosts. For example,
pigs play a role in WN virus persistence (Monath & Heinz, 1990). Furthermore, AROA, IGU, BSQ and NJL viruses, which form a single clade, could be maintained by rodents.

Mosquito-borne viruses normally associated with neurological disease in humans or livestock, leading to encephalitis in severe cases, were found in the Culex clade and were generally associated with viruses that cycled between mosquitoes and birds. DEN virus from the Aedes clades was the exception, since there are rare cases of DEN encephalitis (Lum et al., 1996; Hommel et al., 1998; Solomon et al., 2000). In contrast, the mosquito-borne flaviviruses that are normally associated with haemorrhagic disease were exclusive to the Aedes clades and were associated with viruses that cycle between mosquitoes and primate hosts.

**Clade robustness**

The Culex and Aedes clades showed robust quartet support in the partial E gene phylogenetic tree and robust bootstrap support using the partial NS5 gene sequence for a single Aedes clade containing YF virus. The robustness of the distinction between the Aedes clade that contained the DEN viruses and the Culex clade was separately assessed using a Monte Carlo simulation. The observed phylogeny was subject to 100 Monte Carlo simulations, reconstructed using ML (reconstruction limit = 1) and manually assessed for alternative topologies. Distinct Culex and Aedes clades were observed in 98% of all simulations, while the SPO and ZIK virus sister group formed a trifurcation with the Aedes clade containing DEN virus and the Culex clade in 9% of these simulations.

There are also significant differences between the relative positions of some flaviviruses, as presented in the NS5-derived tree shown in Fig. 1 and those presented by Kuno et al. (1998). For example, ZIK and SPO viruses are positioned together with other Aedes-associated viruses (Fig. 1); they had previously been placed in different positions among the mosquito-borne viruses by Kuno et al. (1998). Secondly, and in contrast with the previous analysis (Kuno et al., 1998), KED virus showed close phylogenetic relationships with SPO and ZIK viruses (Fig. 1), confirming the published serological data (Karabatsos, 1995).

**Tick-borne and NKV flaviviruses**

Vertebrate host clades were also observed in the tick-borne and NKV flaviviruses for both the NS5 and E gene trees. In the NS5 gene phylogeny, NKV viruses, for which APOI virus was the basal lineage, were subdivided into rodent and bat clades. The rodent clade showing robust bootstrap support contained CR, JUT, MOD, SV and SP viruses, whereas the bat clade showing robust bootstrap support contained BUK, CI, DK, PPB and RB viruses (Figs 1 and 2). APOI virus is also associated with rodents and, therefore, could be included in the rodent clade. The bat MML virus was also included in the bat clade, despite low bootstrap support. The remaining three NKV (bat) viruses, ENT, SOK and YOK, in the NS5 gene phylogeny form a sister group with the Aedes clade containing YF virus and are maintained by robust bootstrap support. From the point of view of their evolutionary origins, it is important to note that the rodent clade NKV viruses, with the exception of APOI virus, have only been isolated in the New World, whereas bat-associated viruses have been isolated from the Old and the New World, although none has been isolated in both regions of the world.

The TBE complex viruses were primarily associated with ixodid (hard) ticks, mainly *Ixodes* species and rodent hosts. The second group, consisting of tick-borne seabird-associated viruses (MEA, TYU and SRE), were most frequently isolated from *Ornithodoros* species or *Ixodes uriae*. KAD virus, which is associated with *Rhipecephalus appendiculatus* in Africa and *Hyalomma pratense* in Saudi Arabia, and GGY virus, which is associated with seabirds and *Ixodes uriae*, represent early lineages in the two tick-borne clades and possibly indicate a genetic link between the two tick-borne flavivirus groups.

**Geographical distribution**

The geographical distribution of the mosquito-borne flaviviruses was also examined to see whether or not virus dispersal correlated with either the Aedes clades or the Culex clade. With the exception of YF virus and DEN virus, which are believed to have originated in the Old World but can now also be found in the New World, all other viruses in the Aedes clades are only found in the Old World. On the other hand, the viruses in the Culex clade show geographical clustering, but genetically closely related viruses in the Culex clade have been widely dispersed to the Americas, Africa, Asia and Australasia, i.e. the Old and the New World.

**Discussion**

Early attempts to define flavivirus interrelationships and their evolutionary characteristics were based on antigenic cross reactivity in neutralization, complement fixation and haemagglutination inhibition tests (de Madrid & Porterfield, 1974; Porterfield, 1980; Calisher et al., 1989). Classification schemes based on these criteria have proved helpful in understanding the flaviviruses, but many of the viruses have subsequently been shown to be incorrectly assigned within the schemes. Molecular sequencing and phylogenetic reconstructions have largely overcome these problems and have provided important insights into the taxonomy (Heinz et al., 2000) and dispersal of flaviviruses (Gould et al., 1997). The association of specific flaviviruses with particular arthropod vectors and vertebrate hosts has been defined precisely and a list of these characteristics for each virus is available in the International Catalogue of Arboviruses (Karabatsos, 1995). Despite these extensive data, there have been few previous attempts to correlate molecular evolution with epidemiological
and ecological features of the flaviviruses. The phylogenetic
trees presented here have extended previous analyses of the
flavivirus NS5 (Kuno et al., 1998; Billoir et al., 2000) and E gene
phylogenetic trees (Marin et al., 1995; Zanotto et al., 1995). By
mapping these biological characteristics onto the trees, the
phylogenetic analyses presented in this paper demonstrate a
striking series of correlations between molecular phylogenetic
and ecological/epidemiological characteristics.

It was demonstrated previously (Marin et al., 1995; Kuno et
al., 1998) that the Flavivirus genus was monophyletic and three
distinct groups of viruses, namely tick-borne, mosquito-borne
and NKV viruses, diverge at the deepest nodes. We have now
demonstrated that the mosquito-borne viruses are subdivided
into the Culex clade and Aedes clades. Moreover, the evolution
of the Culex clade appears to have occurred after the separation
of the mosquito-borne viruses from the tick-borne and NKV
viruses. These observations were supported by the congruence
between the NS5 and E gene phylogenies as well as by Monte
Carlo simulation and quartet puzzling support.

The dominance of Aedes and Culex species (subfamily Culicinae) in flavivirus transmission is explained by the species
prevalence of each of the genera, which contain 975 and 769
species, respectively, and comprise more species than all other
mosquito genera combined (1522 species). Aedes and Culex
mosquitoes are also among the small number of genera that are
globally dispersed. Blood-meal data obtained for Aedes species
suggest that mammals are the primary hosts of most species,
which could explain the Aedes clades–primate/mammal
association (Mitchell, 1988; Christensen et al., 1996; Clements,
1999). The feeding patterns of only relatively few species of
Culex mosquito are known, although a small number of bird-
or mammal-specific species have been identified. Many Culex
species feed indiscriminately on both mammals and birds and
they include the principal vectors for several flaviviruses in the
Culex clade, such as C. annulirostris (MVE virus), C. tri-
taeniorhynchus (JE virus), C. tarsalis (SLE virus) and C. unci-
nitatus (WN virus) (Robertson et al., 1993; Christensen et al.,
1996; Clements, 1999). The difference in feeding behaviour
between Aedes and Culex mosquitoes provides a clear
explanation for the associations between Aedes-borne flaviviruses
and mammals or between Culex-borne flaviviruses and birds.
Moreover, it explains why the association between the Aedes
clades and mammals appears to be unequivocal, while the
association between the Culex clade and birds contains a
number of notable exceptions.

The second major correlation was between the type of
disease produced and the mosquito clade in which each virus
appeared. In general, severe infections caused by some Aedes
species viruses result in haemorrhagic disease, whereas many
Culex species viruses cause encephalitic disease; however,
there have been reported cases of DEN (Aedes species-
associated) encephalitis, but these seem to be very rare (Lum et
al., 1996; Hommel et al., 1998; Solomon et al., 2000). Until the
precise basis of flavivirus pathogenicity has been defined at the
molecular level, it is not possible to understand why these
different disease associations can be seen in the phylogenetic
tree. In contrast with the mosquito-borne flaviviruses, different
viruses in the tick-borne virus groups produce encephalitic
disease, but OHF and KFD viruses may also produce
haemorrhagic disease in humans and this does not appear to
correlate with either their phylogenetic or their geographical
characteristics.

Phylogenetic divisions between Old and New World
flaviviruses were seen throughout the NS5 and E gene
phylogenies. In some instances, dispersal of flaviviruses could
be readily linked with the vertebrate host, providing evidence
of the importance of the host in flavivirus evolution. In the case
of viruses that established infections in bats, it is easy to
imagine dispersal to remote sites, as the Old World bats from
which flaviviruses have been isolated are known to migrate
hundreds of kilometres (Shilton et al., 1999). On the other
hand, individual rodent-associated NKV viruses might be
expected to show a more restricted distribution and this is
demonstrated by their detection almost exclusively in the New
World and by their localized or niche-like distribution.

 Virtually all of the tick-borne flaviviruses are exclusively
Old World, with the exception of POW virus. The seabird-
associated tick-borne viruses were dispersed to geographical
areas where they established niches in seabird colonies in both
the Northern and the Southern hemispheres (TYU, SRE and
MEA viruses) (Chastel et al., 1985). At the early period of their
evolution, the TBE complex viruses appear to have been
dispersed either by seabirds or by rodents and their associated
ticks. As they reached the forests of Asia, they became
established predominantly in ixodid species, where they
continued their cline evolution into Europe (Gao et al.,
1993; Zanotto et al., 1995; Gould et al., 1997).

The earliest evolutionary lineages in the mosquito-borne
virus clades appear to have radiated to geographically distant
parts of the Old World and to a wide variety of species, i.e.
bats, Aedes species, sandflies and large animals, including
simians and humans. Only YF virus and the four DEN virus
serotypes, which cause human epidemics, are found in the New
World. There is strong evidence to support the notion that YF
virus was introduced to the Americas from the Old World
during the past few centuries when slaves were transported
across the Atlantic Ocean (Strode, 1951; Monath & Heinz,
1990; Gould et al., 1997).

There are also reasons to believe that DEN viruses have an
African ancestry. The other members of the Aedes clade
containing the DEN, ZIK, SPO and KED viruses were all
isolated from Africa and formed two paraphyletic lineages to
the DEN viruses. In addition, the E gene phylogenies of
demic/epidemic and sylvatic DEN viruses show a basal
position for Old World sylvatic lineages of DEN1, DEN2
(Africa and Malaysia) and DEN4 (Wang et al., 2000). The
vector of DEN virus, Aedes aegypti, is also believed to have
originated in Africa (Tabachnick, 1991). There is no reason to
believe that DEN virus could not have been shipped to the Americas from the Old World in the same way as YF virus. Therefore, as most of the other *Aedes* species-associated viruses are found solely in Africa and since the *Culex* species-associated viruses appear to be descendants of the *Aedes* species-associated viruses, the mosquito-borne flaviviruses appear to have evolved out of Africa.

In conclusion, the flaviviruses that are recognized today represent a diverse group of viruses that could have emerged and dispersed during the past 10,000 years, i.e. since the most recent ice age (Zanotto *et al.*, 1996). The characteristic epidemiological groupings of the viruses that are apparent in the phylogenetic trees illustrate the significant influence of the invertebrate vectors, the vertebrate hosts and the particular ecological niches in which these species have evolved.

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References


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