Acta virol. **35**: 98-105, 1991

ARTHROPOD VECTORS IN THE EVOLUTION OF BUNYAVIRUSES

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Received December 14, 1989

Summary. - Viruses from each genus of *Bunyaviridae* have preferential relationships to the arthropods of only one or two families, i. e. Bunyavirus to mosquitoes (*Culicidae*), Phlebovirus to sand flies (*Psychodidae*) and mosquitoes, Uukuvirus and Nairovirus to ticks (*Ixodidae* and *Argasidae*). An exception is genus Hantavirus not proven to be transmitted by vectors. Within the Bunyavirus genus 16 serogroups have been recognized on the basis of their antigenic relationship. Based on isolations from the nature each serogroup is preferentially linked with arthropod species (mostly mosquitoes) of one, or two genera. For 8 out of 16 serogroups *Culex* mosquitoes are the main insect vectors. Two serogroups are linked with *Aedes* mosquitoes, three with *Anopheles* mosquitoes. *Aedeomyia* mosquitoes, *Culicoides* bitting miges and *Hyalomma* ticks are vectors of one serogroup each. Evolutionary trends within the genus Bunyavirus and within the family *Bunyaviridae* can be recognized based on relationships of bunyaviruses to their arthropod vectors.

Key words: arthropod vectors; bunyaviruses, arboviruses; isolations from nature; evolution

Introduction

The majority of arboviruses has been serologically classified in families *Togaviridae*, *Flaviviridae*, *Reoviridae*, *Bunyaviridae*, and *Rhabdoviridae*, which all possess RNA genomes. Now there are 504 viruses registered in the International Catalogue of Arboviruses and African swine fever is the only DNA virus that is unequivocally considered to be an arbovirus (Karabatsos, 1985).

The families *Togaviridae*, *Flaviviridae*, and *Rhabdoviridae* possess nonsegmented genomes, in which variations arised most probably as a consequence of nucleotide sequence deletions, inversions, and substitutions. Arboviruses with segmented genomes (*Bunyaviridae*, orbiviruses of *Reoviridae*) have even greater evolutionary potential due to their capability of RNA segment reassortment. Two evolutionary mechanisms, intramolecular changes, as well as reassortment, function to promote genetic diversity for segmented viruses (Beaty

et al., 1988). The best example of a successful evolution of viruses with segmented genomes, dispersing into diverse ecological niches with various arthropod and vertebrate hosts, is the family *Bunyaviridae.*

Bunyaviridae genera and arthropods

The *Bunyaviridae* with more than 300 recognized viruses comprises one of the largest families of animal viruses. On the basis of antigenic relationships the family is divided into 5 genera (more than 265 viruses) and about 40 viruses have not been assigned to a genus (Karabatsos, 1985). The viruses of 4 genera (Bunyavirus, Phlebovirus, Uukuvirus, and Nairovirus) are typical arboviruses. There is no evidence for arthropod hosts of the viruses belonging to the fifth genus of the family - Hantavirus (Lee, 1982).

Despite of the variety of vertebrate hosts and arthropod vectors which can be infected with arboviruses, each arbovirus shows a remarkable specificity with respect to its natural hosts, infecting only one or a small number of vertebrate and arthropod species (Chamberlain, 1980), representing the specific ecological niche for an arbovirus. This ecological niche is influenced by many factors, but is predetermined by trophic relationships of the arthropod vectors to the selected vertebrates.

It is well known, that in nature each individual arbovirus of the family *Bunyaviridae* infects strikingly limited number of arthropod hosts (Murphy *et al.*, 1975; McClintock, 1978). Moreover, the viruses of each genus of *Bunyaviridae* have the preferential relationships to the arthropods of only one or two families. From the data collected in the International Catalogue of Arboviruses (Karabatsos, 1985) and from a number of other published papers more than

Table 1. Participation of arthropod families in the isolations of bunyaviruses of different genera in nature

Arthropod family	*Bunyaviridae*			
	Bunyavirus	Phlebovirus	Uukuvirus	Nairovirus
Culicidae	89.4 %	?	-	0.3 %
Ceratopogonidae	10.3 %	-	-	0.4 %
Psychodidae	0.1 %	(99.7 %)	-	-
Ixodidae	0.1 %	-	75.6%	82.7 %
Argasidae	-	-	24.4 %	16.3 %
Other	0.1 %	-	-	0.3 %
No. of viruses per No. of strains*	114/3515	(21/380)	6/119	21/719

*Based on data from International Catalogue of Arboviruses (Karabatsos, 1985)
?=mosquito-borne phleboviruses are missing, no exact numbers available

5000 strains of viruses of Bunyavirus, Phlebovirus, Uukuvirus, and Nairovirus genera have been summarized with regard to the isolations from arthropods (Table 1).

The largest genus of the *Bunyaviridae* family is genus Bunyavirus with 129 registred and more than 153 recognized viruses (Calisher and Karabatsos, 1988); 114 viruses of the genus registred in the Catalogue have been isolated from arthropods (about 3500 strains) and almost nine tenth (89.4%) of strains have resulted from mosquitoes (*Culicidae*). Typical insect hosts for 21 viruses of the Phlebovirus genus are sand flies *(Phlebotominae, Psychodidae*). Almost 100 % of about 400 strains of these viruses have been isolated from them. Moreover, 4 viruses of Phlebovirus genus are frequently isolated from mosquitoes, among them Rift Valley fever virus for many times. Almost 100% of isolations of these viruses have been from mosquitoes (data of these 4 viruses are not included in the Table 1).

Ticks are the main arthropod hosts of viruses of the genus Uukuvirus and Nairovirus. The six registered viruses of the genus Uukuvirus have been isolated from arthropods about 120 times and all the isolates are from ticks. Out of them, 73.9% are from ticks of genus Ixodes (*Ixodidae*) and 24.4% from genus Argas (*Argasidae*). Tick-borne are also the 21 registred viruses of genus Nairovirus, more than 700 strains having been isolated from arthropods, mostly from ticks (99%) belonging to various genera of *Ixodidae* (82.7%) and *Argasidae* (16.3%).

Table 2. Serogroups of the genus Bunyavirus in relation to the genus of the main arthropod hosts expressed as the percentual participation of arthropod genus in the isolations from nature

Serogroup	No. of viruses / No. of strains*	Arthropod genus	%
Capim	7/ 61	*Culex*	100
C group	13/ 267	*Culex*	92.9
Guama	9/ 324	*Culex*	94.7
Minatitlan	1/ 3	*Culex*	100
Patois	5/ 92	*Culex*	98.9
Olifantsvlei	4/ 6	*Culex*	83.3
Koongal	2/ 67	*Culex*	94.0
Turlock	4/ 300	*Culex*	100
Gamboa	3/ 44	*Aedeomyia*	100
California	13/ 472	*Aedes*	86.7
Bwamba	2/ 57	*Aedes*	73.7
Anopheles A + B	7/ 24	*Anopheles*	54.2
Bunyamwera	24/ 1545	*Anopheles*	47.6
Simbu	18/ 250	*Culicoides*	84.0
Tete	2/ 3	*Hyalomma*	100

*No. of viruses isolated from arthropods/No. of strains of these viruses isolated from arthropods

Relationships among Bunyaviridae genera

The bunyaviruses have been divided into five genera on the basis of their antigenic relationships. Members of each genus do not share antigenic relationship with members of the other four genera (Calisher and Karabatsos, 1988). The genome of the *Bunyaviridae* consists of three unique, single stranded RNA segments of negative (or ambisense) polarity enclosed in the spherical and enveloped virions, 90 to 100 nm in diameter. Extensive studies on the molecular biology of bunyaviruses in recent years demonstrated similar, but distinctive coding strategies for the five genera of *Bunyaviridae.*

Briefly, each bunyavirus virion RNA segment has a conserved RNA sequence with inverted complementarity of 8–13 nucleotides at the 3' and 5' termini. The conserved region is distinct for each genus, although very similar for Uukuvirus and Phlebovirus where the first 8 bases are identical (Gonzales-Scarano and Nathanson, 1989).

The large (L) RNA segment codes for the large protein which is viral RNA polymerase (Endres *et al.*, 1989). The medium (M) RNA segment codes for two glycoproteins (G 1 and G 2) and, in Bunyavirus and Phlebovirus genera, also for a nonstructural protein. This segment contains a single long open reading frame (ORF), which is translated as a polyprotein and cleaved into proteins in the order in which nonstructural protein is between structural proteins in the genus Bunyavirus and at the 5' end in the genus Phlebovirus. The segments of Uukuvirus and Hantavirus are ordered 5' - G 1 - G 2-3' with no nonstructural protein. The sequence of the (M) RNA segment of Uukuvirus showed homology with the genus Phlebovirus but none with the genus Bunyavirus (Rönnholm and Pettersson, 1987). The small (S) RNA segment codes for a nucleocapsid (N) protein and in some genera also for a nonstructural protein; the latter is encoded in a different ORF than the N protein and in different genera it follows a different coding strategy namely overlapping ORFs in Bunyavirus, but an ambisence coding strategy in Phlebovirus (Gonzales-Scarano and Nathanson, 1989).

It is attractive to speculate whether and how the differences in the structure of viral genome influence the selection of host species of different arthropod families, and vice versa. Obviously both, the coding strategies and arthropod hosts specificity are results of evolutionary trends within the *Bunyaviridae.*

Bunyavirus serogroups and mosquitoes

On the basis of antigenic relationships each genus of *Bunyaviridae* forms one or more serogroups. There are 16 serogroups recognized within the genus Bunyavirus. Based on isolations from nature each serogroup is preferentially linked together with arthropod (mostly mosquito) species of one, or two genera. Many arthropods are involved in isolations of bunyaviruses, but the main vectors of Bunyavirus genus belong to only 6 genera (Table 2).

For 8 out of 16 serogroups are mosquitoes of the genus *Culex* the main insect hosts (Fig. 1). The relationship to *Culex* mosquitoes is highly specific. Isola-

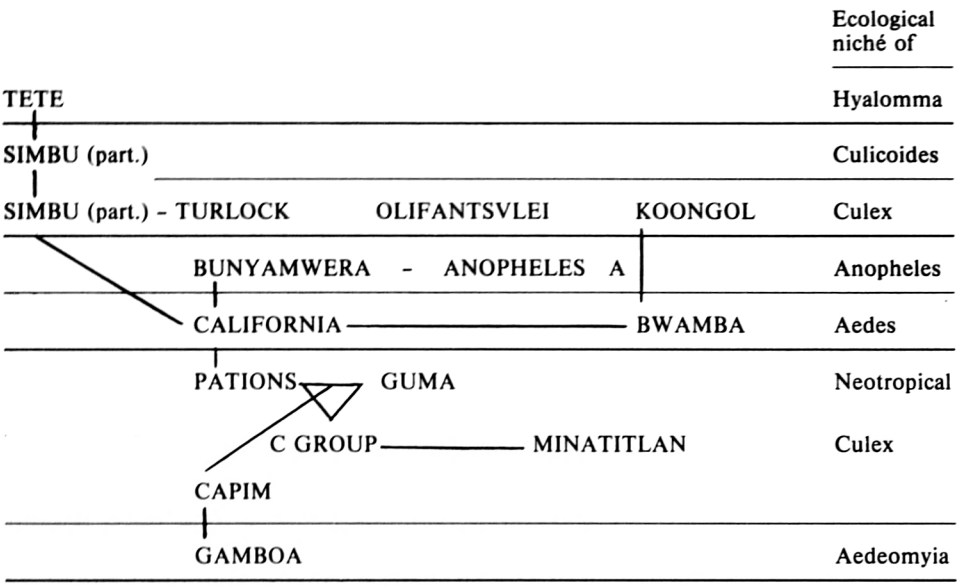

Fig. 1
Antigenic relationships among Bunyavirus serogroups (modified from Calisher, 1988) and ecological nichés represented by arthropod vector genera (from these about a half or more strains have been isolated)

tions of viruses of these serogroups from other than *Culex* mosquitoes are rare (only 4.0% out of 1120 isolated strains). Out of 8 serogroups transmitted by *Culex* mosquitoes 5 serogroups are geographically distributed in Neotropical region (southern parts of North America, Central and South America) and are antigenically closely related to each other (Calisher, 1988). In total 35 viruses have been recognized in these 5 serogroups, and 747 strains have been isolated from insects, mostly from *Culex* mosquitoes of subgenus Melanoconion. Only 39 strains of only 13 viruses have been isolated from other than *Culex* mosquitoes, but they belong to 6 mosquito genera and two strains have been isolated also from Lutzomyia sand flies (*Psychodidae*). For me it demonstrates, besides the high specificity to *Culex* mosquitoes, the ability of these viruses (e. g. Guama and Oriboca viruses) to invade new ecological niches with new vectors, which feed on different spectrum of vertebrate species. In new ecological niches selective pressures force new evolutionary trends leading to the differentiation of novel Bunyavirus serogroups.

Antigenically related to these viruses across Capim serogroup are viruses of Gamboa serogroup, which are distributed in the same geographic region.

These viruses are transmitted exclusively by *Aedeomyia* mosquitoes (Calisher *et al.*, 1988).

Intramolecular changes and RNA reassortment are the main evolutionary mechanisms for diversity of these viruses. Bunyavirus genome reassortment in dually laboratory infected mosquitoes has been observed and it was successful only in mosquitoes (Beaty *et al.*, 1985). Natural RNA reassortment was demonstrated for viruses of C group (Shope and Causey, 1962). The attractive idea is that the viruses of Neotropical serogroups differentiated from a comon ancestral Bunyavirus, or Bunyavirus serogroup. The period of South American geographical isolation with diversity of endemic insect (subgenus Melanoconion of *Culex* mosquitoes), bird and mammal (marsupials) species could form favourable conditions for introduction of new group of RNA viruses, such as bunyaviruses. Later on, the radiation of bunyaviruses into diverse ecological niches with different mosquito species and genera could be the main source of evolution. As the result of natural selection in the changed environment of new insect and vertebrate species new serogroups could emerge.

The other three serogroups with *Culex* mosquitoes as the main arthropod hosts are Olifantsvlei from Africa, Koongal from Australia and Turlock with large distribution over Americas, Europe, Africa, and Asia. They are antigenically unrelated to each other and to the Neotropical viruses as well.

The antigenic relationship to the Neotropical serogroups was demonstrated for California serogroup, across the relationship with Patois serogroup.

The main arthropod hosts for largely over Americas, Africa, Europe, and Asia, distributed California serogroup viruses are mosquitoes of the genus *Aedes*. The largely distributed representative of California serogroup in Europe is Ťahyňa virus. Here it has been isolated from 14 mosquito species of 5 genera (Pilaski and Mackenstein, 1985). Out of 94 analysed Ťahyňa virus strains 88.3% have been isolated from 8 *Aedes* species and half of them (40.4%) have been from the main vector *Aedes vexans* (Labuda and Kožuch, 1982; Pilaski and Mackenstein, 1985). *Aedes* mosquitoes are also hosts of African Bwamba serogroup viruses, which are antigenically related to California serogroup. *Aedes* mosquitoes as vectors are not determined for all viruses of the California serogroup. E. g. Guaroa virus from California serogroup is transmitted by *Anopheles* mosquitoes. Interestingly, across this virus is the California serogroup antigenically related to the Bunyamwera serogroup, which is transmitted mostly by *Anopheles* mosquitoes. The role of *Anopheles* mosquitoes as vectors for Bunyamwera serogroup viruses is also not always clearly stressed. E. g. North American Main Drain and Tensaw viruses are transmitted with *Culicoides* biting midges (*Ceratopogonidae*).

Culicoides biting midges are the vectors of viruses of largely distributed Simbu serogroup. This serogroup can be split into viruses transmitted almost exclusively with *Culicoides* and viruses transmitted exclusively with *Culex* mosquitoes. Viruses of the last Tete serogroup have been in three occasions isolated from *Hyalomma* ticks in North Africa. These three strains are the only

Bunyavirus isolates from ticks, it is difficult to discuss the significance of ticks for Tete serogroup until more data will be available.

The antigenic relationships among Bunyavirus serogroups with their ecological niches are demonstrated in Fig. 1. There are clear parallels between antigenic relationships (crossreactions among serogroups) and relationships to the arthropods within the genus Bunyavirus. In my opinion, arthropod vectors, or better to say arthropod hosts are largely responsible for antigenic properties of bunyaviruses. We can speculate that the evolutionary trend within the genus Bunyavirus have radiated from highly specific relationship with *Culex* mosquitoes and limited geographic distribution to the less specific virus-vector relationships with *Aedes* and *Anopheles* mosquitoes and large geographic distribution.

The specific relationships with arthropod vectors are not unique for negative-stranded RNA viruses of the family *Bunyaviridae*. Positive-stranded RNA viruses of the genus Alphavirus (family *Togaviridae*) are transmitted exclusively by mosquitoes. Positive-stranded RNA viruses of the genus Flavivirus (family *Flaviviridae*) are transmitted by both, mosquitoes and ticks. Antigenically, tick-borne flaviviruses are closely related to each other and are separated from mosquito-borne flaviviruses.

The evolution of RNA viruses has received a great deal of attention in the past several years. Complete nucleotide sequence of the genomic RNAs of a number of viruses belonging to different virus families have been obtained, and these make possible detailed comparisons of the families on a molecular level. These comparisons have led to new insights into RNA virus evolution and taxonomy, including a better understanding of the radiation of viruses towards different hosts. On the basis of sequence similarities and higher order similarities Strauss and Strauss (1988) hypothesized that all the positive-stranded RNA viruses, i.e . those that contain the message sense RNA as their genome, have evolved from a common ancestor. Further, all of the currently known negative-stranded viruses, appear to form a homogeneous group, which suggests that they too descended from a common ancestor.

The positive-stranded insect viruses with two genome segments of the family *Nodaviridae* share the characteristics of both plant and animal viruses, which has led to the suggestion that the prototype positive-strand virus originated in insects (Strauss and Strauss, 1988). The negative-stranded viruses with three genome segments of the family *Bunyaviridae* probably originated also in inscets (at least judging from the intimate association of these viruses with mosquitoes). It remains to be seen whether the positive and negative-stranded RNA viruses also shared a common ancestor in the past.

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