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# Molecular and Biochemical Studies of the Evolution, Infection and Transmission of Insect Bunyaviruses

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# Molecular and biochemical studies of the evolution, infection and transmission of insect bunyaviruses

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Members of the Bunyaviridae family of RNA viruses (bunyaviruses, hantaviruses, nairoviruses, phleboviruses and uukuviruses) have been studied at the molecular and genetic level to understand the basis of their evolution and infection in vertebrate and invertebrate (arthropod) hosts. With the exception of the hantaviruses, these viruses infect and are transmitted by a variety of blood-sucking arthropods (mosquitoes, phlebotomines, gnats, ticks, etc.). The viruses are responsible for infection of various vertebrate species, occasionally causing human disease, morbidity and mortality (e.g. Rift Valley fever, Crimean–Congo haemorrhagic fever, Korean haemorrhagic fever). Genetic and molecular analyses of bunyaviruses have established the coding assignments of the three viral RNA species and documented which viral gene products determine host range and virulence. Ecological studies, with molecular techniques, have provided evidence for bunyavirus evolution in nature through genetic drift (involving the accumulation of point mutations) and shift (RNA-segment reassortment).

## 1. Introduction

This paper discusses the evolution and genetic determinants of disease and infection by representatives of one group of arthropod-transmitted viruses, the bunyaviruses (*Bunyavirus* genus, Bunyaviridae family) (Bishop *et al.* 1980; Matthews 1982). This group of arboviruses has been selected because of its diversity (some 160 different viruses have been described, see Karabatsos (1985)) and because it has been possible to use both genetic and molecular tools to study the infection processes in permissive vertebrate and invertebrate hosts.

Arboviruses such as bunyaviruses are transmitted indirectly by inoculation into vertebrates by haematophagous insects. This route of transmission differs from that of viruses that are passed directly or incidentally between two hosts (e.g. the common cold viruses, which are spread by aerosols, or the enteric polio viruses, which are spread by contamination involving the faecal–oral route). Bunyaviruses replicate in both the permissive arthropod species and the vertebrate host. In this regard they differ from viruses that are mechanically transmitted by insects. Bunyaviruses do not appear to cause overt damage to the arthropod vector and so are unlike, for example, the directly transmitted insect-specific baculoviruses which characteristically are pathogenic for the larval forms of particular arthropod species (e.g. caterpillars). Because arthropod-borne viruses are injected into a vertebrate when an insect takes a blood meal, or are acquired from an infected verebrate when an insect feeds, the viruses do not have to survive outside a living organism.

Virus evolution has to be considered in relation to the opportunities afforded for selection and biological cloning in nature. This paper describes the information that is available

concerning bunyavirus evolution in relation to the coding strategies of its RNA species and the molecular and genetic determinants of infection by bunyaviruses in both arthropod and vertebrate hosts.

## 2. The Bunyaviridae

The hierarchy of relationships that has been proposed for members of the Bunyaviridae is illustrated in table 1 (Bishop 1985). Viruses are classified in subfamilies, genera and serogroups. Five genera of viruses have been recognized: *Bunyavirus*, *Phlebovirus*, *Nairovirus*, *Uukuvirus* and *Hantavirus* (Bishop *et al.* 1980; McCormick *et al.* 1982; White *et al.* 1982; Schmaljohn & Dalrymple 1983). The diversity that exists among the members of the Bunyaviridae includes differences in both host and vector preferences (Karabatsos 1985). Viruses of this family have been obtained from every continent of the world except Antarctica. Most of the viruses have only been isolated from a limited number of arthropod species (e.g. certain *Aedes* species). However, only in some cases have such insects been shown to be permissive for virus transmission. Haematophagous insects acquire virus when they take an infected blood meal. Some may not be permissive for virus replication at all. The identification of viruses in such insects is incidental to the normal cycle of virus maintenance in nature. In other species there may only be a low level of virus replication in the requisite insect tissues (e.g. their salivary glands) so that the efficiency of transmission is limited (such insects therefore may be considered as semi-permissive). When virus replication is restricted still further (e.g. only to insect mid-gut tissues) there may be no opportunity for virus transmission to a vertebrate host. The molecular determinants of the permissiveness of particular cells, or organs, in an arthropod host for virus replication are not known.

For many members of the Bunyaviridae certain warm-blooded vertebrates have been shown to be amplifying hosts (Karabatsos 1985) (e.g. man, domestic animals and wildlife). The virus replicates in such hosts, thereby allowing a blood-sucking insect to become infected. The vertebrate hosts may also aid in virus dissemination by virtue of their lifestyles (e.g. through their migration). Obviously a virus may be introduced, on occasion, into vertebrates that are not its usual host(s). In some of these hosts there may be no virus replication and little or no immunological response. In others, the virus may replicate to only a limited extent, insufficient to produce a high-titred viraemia. The infection of vertebrates reflects the host preferences of the insect species and the ability of the virus to replicate in cells of those vertebrates. Where an occasional introduction into a vertebrate leads to a transient virus infection, and where that vertebrate is the preferred target of another species of blood-sucking insect, there is opportunity for the virus to infect a different arthropod species. Without doubt, the existence of such opportunities has contributed to the evolution and diversity seen among the Bunyaviridae both in terms of virus isolation and the preferred vector and host species.

Some members of the Bunyaviridae family (e.g. the aetiological agent of Korean haemorrhagic fever, Hantaan virus) do not have an arthropod vector. Although for other members of the family no vertebrate host has been identified by virus isolation, serology, or other surveys (Karabatsos 1985), this circumstance probably reflects the lack of identification of, rather than an absence of, a vertebrate host.

As discussed later, horizontal transmission of virus has been documented for several bunyaviruses. Such transmission includes the acquisition of virus by an insect feeding on




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Table 1. Proposed hierarchy of Bunyaviridae relationships

| | | | number of viruses |
|---|---|---|---|
| subfamily: | Bunyavirinae | | |
| | genus | *Bunyavirus* | |
| | serogroups | Anopheles A group | 12 |
| | | Anopheles B group | 2 |
| | | Bunyamwera group | 32 |
| | | Bwamba group | 2 |
| | | C group | 14 |
| | | California group | 14 |
| | | Capim group | 10 |
| | | Gamboa group | 8 |
| | | Guama group | 12 |
| | | Koongol group | 2 |
| | | Minatitlan group | 5 |
| | | Olifantsvlei group | 5 |
| | | Patois Group | 6 |
| | | Simbu group | 24 |
| | | Tete group | 5 |
| | | Turlock group | 5 |
| | | ungrouped | 2 |
| subfamily: | Phlebovirinae | | |
| | genus | *Phlebovirus* | |
| | serogroups | Sandfly fever Naples group | 4 |
| | (complexes) | Bujaru group | 2 |
| | | Candiru group | 6 |
| | | Chilibre group | 2 |
| | | Frijoles group | 2 |
| | | Punta Toro group | 2 |
| | | Rift Valley fever group | 3 |
| | | Salehabad group | 2 |
| | | unassigned | 16 |
| | genus | *Uukuvirus* | |
| | serogroup | Uukuniemi group | 12 |
| subfamily: | Nairovirinae | | |
| | genus | *Nairovirus* | |
| | serogroups | Crimean–Congo haemorrhagic fever group | 3 |
| | | Dera Ghazi Khan group | 6 |
| | | Hughes group | 10 |
| | | Nairobi sheep disease group | 2 |
| | | Qalyub group | 3 |
| | | Sakhalin group | 7 |
| subfamily: | Hantavirinae | | |
| | genus | *Hantavirus* | |
| | serogroup | Hantavirus | 8 |
| | probable (possible) viruses not assigned to a subfamily or genus | | |
| | serogroups | Bakau group | 4 |
| | | Bhanja | 3 |
| | | Kaisodi group | 3 |
| | | Mapputta group | 4 |
| | | Matariya group | 3 |
| | | Nyando | 3 |
| | | Okola | 2 |
| | | Yogue group | 2 |
| | | unassigned | 27 |




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infected blood, or an infected arthropod inoculating a vertebrate host, or an infected male insect inseminating and infecting a female insect. Vertical transmission of virus in arthropods has been demonstrated for some bunyaviruses. The *in ovo* transmission of virus is one way in which a virus may overwinter and persist in the environment.

### 3. Bunyavirus genus

Serological tests have been used to define the antigenic relationships of members of the Bunyaviridae (Bishop & Shope 1979; Karabatsos 1985). They suggest that viruses can sensibly be grouped into genera. For the most part cross-neutralization of infectivity, cross-haemagglutination inhibition, competition radioimmune precipitation, cross-complement fixation and immunodiffusion tests have been used to establish the serogroup relationships presented table 1. In general, a member of a *Bunyavirus* serogroup is neutralized by homologous antisera and (with different specificities) by antisera raised to other members of that serogroup (Bishop & Shope 1979). A virus in one of these serogroups is not neutralized by antisera raised to members of another bunyavirus serogroup. Haemagglutination-inhibition (HI) studies have given similar results. For bunyaviruses the data from neutralization and HI tests reflect the relationships of the viral glycoproteins. Other serological tests, cross-complement fixation studies, competition radioimmune precipitation and immunodiffusion analyses have revealed relationships involving members of different serogroups (see Klimas *et al.* 1981*b*). This is because such tests measure the antigenic relationships of the more conserved, internal, antigens of bunyaviruses (e.g. the viral nucleoproteins). For this reason the various bunyavirus serogroups have themselves been grouped together into the *Bunyavirus* genus.

Similar observations have been made in analyses of members of the other genera of the Bunyaviridae (Bishop & Shope 1979). In comparison with the bunyavirus results there are differences in the usefulness and applicability of the various serological tests employed to characterize the relationships of the members of these other genera.

Members of a Bunyaviridae genus (e.g. the *Bunyavirus* genus) do not share antigenic epitopes

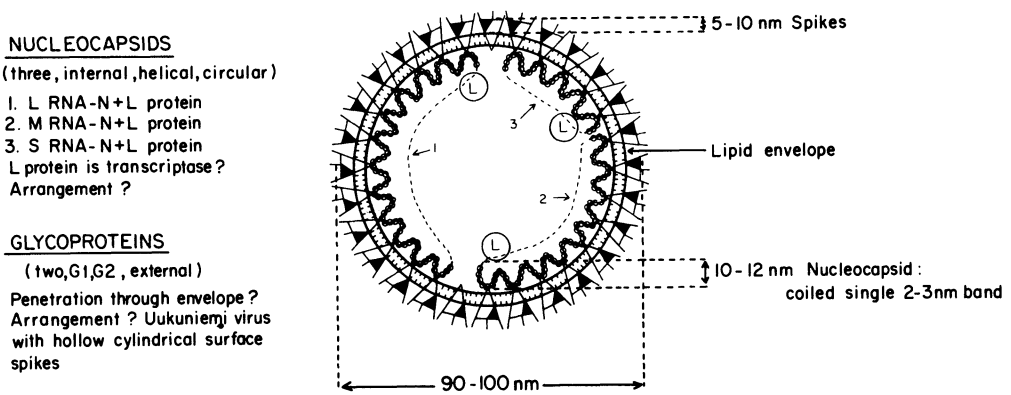


Figure 1. Schematic Bunyaviridae virus particle. Viruses are spherical (approximately 100 nm in diameter), enveloped in lipid that is derived from the Golgi membranes of infected cells, with an outer fringe of glycoprotein (e.g. G1, G2) and inner components including three nucleocapsids consisting of RNA (L, M, S), a major structural protein (N) and minor quantities of a large protein (L) that is believed to be a transcriptase. Although apparently circular, the RNA species in the nucleocapsids have complementary 3′ and 5′ end sequences that may be responsible for holding the structures in the circular configuration.

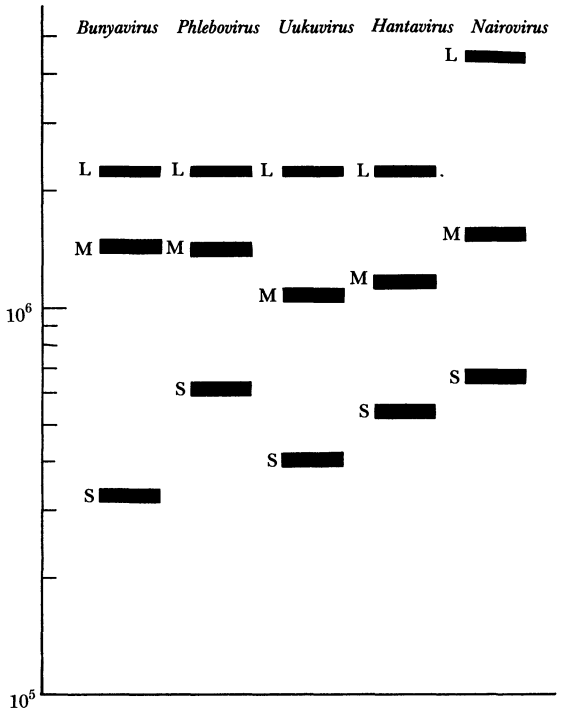


FIGURE 2. Consensus sizes of the viral RNA species of bunyaviruses, uukuviruses, phleboviruses, hantaviruses and nairoviruses.

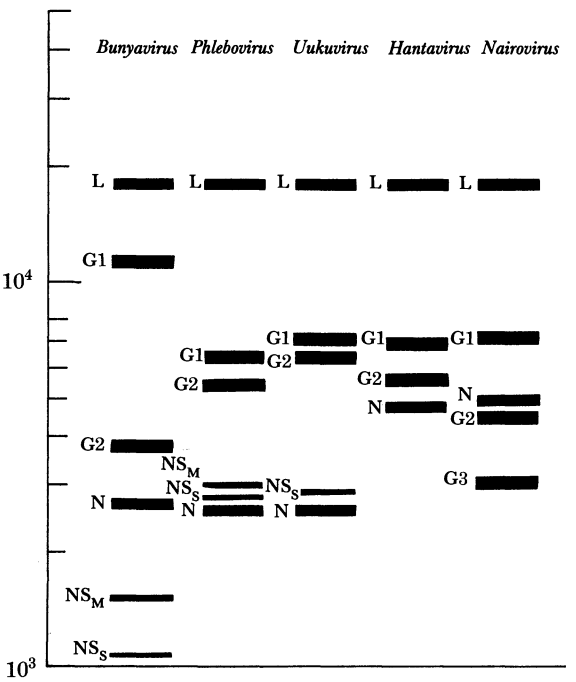


FIGURE 3. Consensus sizes of the viral protein species of bunyaviruses, uukuviruses, phleboviruses, hantaviruses and nairoviruses.




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with members representing another genus (e.g. the *Hantavirus* genus). In addition, members of the various genera, although they are similar in shape (figure 1), have distinctly sized RNA (figure 2) and protein (figure 3) species (Bishop 1985). The limited RNA-sequence analyses that have been reported support the assignments and separation into genera shown in table 1.

## 4. Structural characteristics of bunyaviruses

Like other members of the family, bunyaviruses are enveloped, spherical and approximately 90–100 nm in diameter (Bishop & Shope 1979) (figure 1). Virus infectivity is readily destroyed by treatment with lipid solvents (as with all enveloped viruses). Virus particles contain three internal nucleocapsids each consisting of viral nucleoprotein (N), a unique single-standard species of RNA and transcriptase enzyme. The N protein has group-specific antigenic determinants. Viruses have an outer surface layer of glycoproteins (e.g. bunyaviruses have glycoproteins G1, G2) on which are located the type-specific antigens.

## 5. The infection process of bunyaviruses

The molecular events involved in virus replication have been defined only in broad outline for a few members of the family, notably certain bunyaviruses and phleboviruses (Bishop 1985). Bunyavirus replication has been reported to occur in the cytoplasm of infected cells (Goldman *et al.* 1977). After adsorption and penetration involving viral phagocytosis, the nucleocapsids are released into the cell cytoplasm and viral mRNA synthesis is initiated (primary transcription) (Vezza *et al.* 1979; Eshita *et al.* 1985). Short (12–17 nucleotides long) non-viral sequences have been identified at the 5′ termini of the bunyavirus mRNA species (Bishop *et al.* 1983*b*; Eshita *et al.* 1985). Presumably the non-viral sequences are acquired by the viral transcriptase from RNA species of the host cell and used to prime the synthesis of viral mRNA (Patterson *et al.* 1984). Overall, however, the mRNA species are shorter than their corresponding viral RNAs owing to the fact that mRNA transcription terminates before the 3′ end of the template RNA is reached (Eshita *et al.* 1985). It is not known what signals transcription termination.

It appears that bunyaviruses have a simple negative-standard coding arrangement of their L, M and S RNA species with proteins translated from viral-complementary mRNA sequences (Clerx-van Haaster & Bishop 1980; Bishop *et al.* 1983*a*; Eshita & Bishop 1984; Fields 1985). The N and a non-structural protein ($NS_S$) of bunyaviruses are translated from overlapping reading frames of the S mRNA species (Bishop *et al.* 1983*a*) (figure 4). No splicing of mRNA has been demonstrated by sequence analyses of the bunyavirus S mRNA species (Bishop *et al.* 1982, 1983*b*). An unanswered question is what, if anything, regulates the reading of the two gene products from the single bunyavirus S mRNA species.

The M RNA of bunyavirus codes for the two viral glycoproteins and a second non-structural protein ($NS_M$). These proteins are made as a single precursor molecule translated from the viral-complementary mRNA sequence (Eshita & Bishop 1984). The function of the $NS_M$ protein and its location in an infected cell are unknown. The M coded mRNA species has a 5′ terminus consisting of a small non-viral sequence and a 3′ end that is some 100 nucleotides shorter than the viral RNA (Eshita *et al.* 1985). The coding strategy of the bunyavirus L RNA




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has yet to be reported. It is believed to code for the virion L protein, the candidate transcriptase–replicase of bunyaviruses.

It is assumed that, after primary transcription, the mRNA species are translated by the cellular machinery. With the availability of new gene products, viral RNA replication then commences (see figure 4). Secondary transcription, dependent on RNA replication, results in the synthesis of larger amounts of the viral mRNA species and proteins (Vezza *et al.* 1979). This is followed by the onset of viral morphogenesis which usually involves the budding of virus

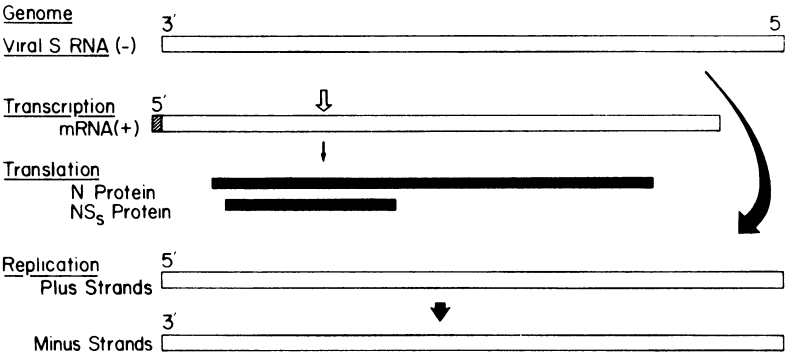


FIGURE 4. Coding, transcription and replication strategies of the bunyavirus S RNA species. Transcription of the single S coded mRNA from the negative sense (−) viral RNA species involves the use of primers (stippled) to initiate mRNA synthesis. The S mRNA codes for N and a non-stuctural protein ($NS_s$) in overlapping reading frames. Replication of the viral S RNA involves the production of a complete viral-complementary intermediate to the synthesis of viral S RNA.

particles into the cisternae of a cell's Golgi apparatus (Murphy *et al.* 1968; Smith & Pifat 1982). Virus particles can often be visualized in intracytoplasmic vesicles. Presumably the reason why morphogenesis of bunyaviruses is usually restricted to the Golgi system of a cell is because the viral glycoproteins are not transported to the outer plasma membrane of the cell. The reason for this is unknown. Viral egress from infected cells is assumed to involve fusion of the vesicles containing virus particles with the cell surface membrane followed by release of the virions (Smith & Pifat 1982). Usually during infection there is a virus-induced proliferation of the cisternae of the Golgi apparatus.

## 6. BUNYAVIRUS EVOLUTION BY GENETIC DRIFT

Evidence has been presented that for La Crosse (LAC) bunyavirus (California serogroup, table 1) no two virus isolates recovered from nature have identical genome sequences as evidenced by RNA oligonucleotide fingerprinting (figure 5), or by RNA sequencing (El Said *et al.* 1979; Klimas *et al.* 1981*a*; Clerx-van Haaster *et al.* 1982). This observation applies to viruses isolated from the same place but at different times, or at the same time but different places. However, by such procedures most of the LAC virus isolates have been shown to be closely related to each other (although they are also distinguishable). The relationships that have been seen are taken as evidence for the continuous evolution of the virus by the accumulation of point mutations.




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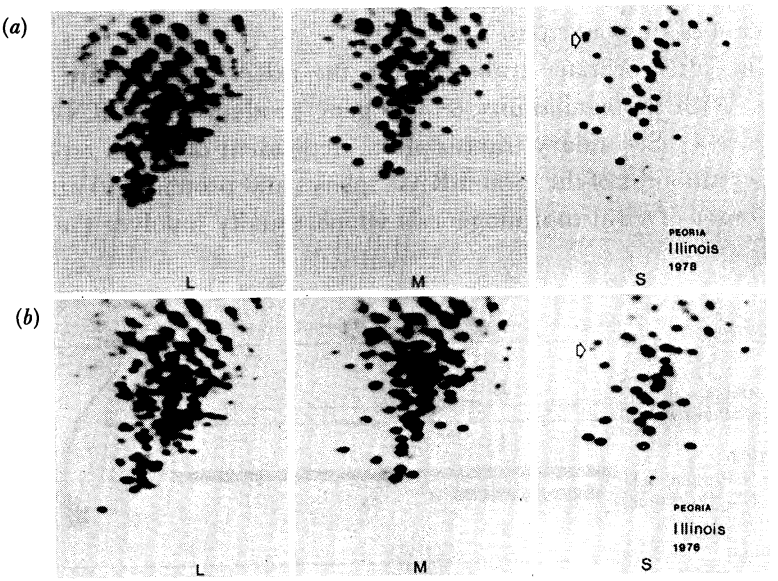


FIGURE 5. Oligonucletde fingerprint analyses of two La Crosse virus isolates obtained from mosquitoes collected at the same site in Peoria, Illinois, but at different times. (*a*) 1978; (*b*) 1976. (See Klimas *et al.* (1981 *a*) for further examples.)

## 7. BUNYAVIRUS EVOLUTION BY RECOMBINATION (REASSORTMENT)

In view of the observation that bunyaviruses have a tripartite RNA genome with segments coding for different gene products, the possibility that recombinant viruses may be generated in dual virus infections by RNA-segment reassortment (or by other mechanisms) has been investigated. Genetic studies using temperature-sensitive (*ts*) mutants of snowshoe hare (SSH) bunyavirus (California serogroup, table 1) have confirmed that intratypic wild-type recombinant viruses can be formed from *ts* mutants representing different RNA species (Gentsch & Bishop 1976; Gentsch *et al.* 1977; Gentsch *et al.* 1979). Similar results have been reported for LAC, Tahyna, trivittatus, Lumbo and other California group bunyaviruses (Bishop & Shope 1979). For example, on co-infection of cells with LAC *ts* I-16 (an M RNA mutant) and LAC *ts* II-5 (an L RNA mutant), recombinant wild-type viruses were recovered that, unlike the parent *ts* viruses (which only gave plaques at 33 °C), produced plaques at both 39.8 and 33 °C.

Intertypic genetic recombination has been demonstrated by using *ts* mutants representing different California serogroup viruses (Gentsch *et al.* 1977, 1979, 1980; Gentsch & Bishop 1978, 1979; Rozhon *et al.* 1981; Shope, *et al.* 1981). Using SSH and LAC *ts* mutants, all the possible genotypes of reassortant viruses ($2^3$, i.e. 8) have been isolated from dual virus infections. Reassortant viruses have been obtained between LAC, SSH, California encephalitis, trivitattus, Lumbo and Tahyna viruses (all members of the California group). Similar data have been reported for Bunyamwera serogroup members (Iroegbu & Pringle 1981 *a*, *b*; Pringle & Iroegbu 1982) and group C bunyaviruses (Bishop *et al.* 1983 *a*). However, viruses representing different bunyavirus serogroups do not appear to be capable of generating recombinant viruses, and also not all viruses assigned to a single bunyavirus serogroup are genetically interactive (see Pringle *et al.* 1984). No reassortants have been detected from co-infections involving the above California serogroup members and Guaroa virus (a serologically distant




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California group virus), Oriboca or Caraparu (group C) viruses, or with several members of the Bunyamwera serogroup. Whether this conclusion extends to all bunyavirus serogroups, or to members of serogroups of other genera, remains to be determined.

A question that arises from these *in vitro* experimental observations is whether bunyaviruses in their natural environment evolve through genetic recombination (reassortment). This question has to be considered in relation to the preferred vertebrate and invertebrate hosts, the viral determinants of permissive infections, and the opportunities afforded for dual virus infection (see below). Direct evidence for naturally occurring reassortant viruses has been obtained. RNA genome fingerprint analyses or field isolates of LAC virus have provided evidence for intratypic recombinant (reassortant) LAC viruses (Klimas *et al.* 1981*a*). Reassortant viruses have also been identified among field isolates of members of the Patois serogroup of bunyaviruses (Ushijima *et al.* 1981).

## 8. Bunyavirus infections of mosquito species

The natural relation between LAC virus and *Aedes triseriatus* mosquitoes has been extensively studied. This mosquito species has been demonstrated to be an efficient oral (Watts *et al.* 1972), transovarial (Pantuwatana *et al.* 1974; Watts *et al.* 1973*a*, *b*) and venereal transmitter of LAC virus (Thompson & Beaty 1977). In temperate regions of the United States of America, LAC virus overwinters in diapaused *Ae. triseriatus* eggs (Watts *et al.* 1974; Beaty & Thompson 1975).

Immunofluorescence techniques have been used to determine the virogenesis of LAC in *Ae. triseriatus* and to derive anatomical explanations of the vector–virus interactions (transovarial and venereal transmission) observed in this system. After oral infection, virus antigen was first detected in the pyloric portion of the midgut (six days post-infection). By ten days the virus had disseminated from the midgut and antigen was detected in most secondary organ systems, including ovaries and salivary glands. It was observed that LAC virus infection was virtually pantropic in the arthropod with most organ systems containing large quantities of virus antigen. Detection of virus antigen in ovarian follicles and in accessory sex-gland fluid provided anatomical explanations for the observed transovarial and venereal transmission, repectively (Beaty & Thompson 1976, 1977).

Although serologically closely related, each of the California serogroup bunyaviruses has a distinct epizootiology often involving particular, but not exclusive, vector and vertebrate hosts (Turell & LeDuc 1983). For example, in the United States of America trivittatus virus is closely associated with an *Ae. trivittatus* – cottontail rabbit feeding cycle. Keystone virus is associated with an *Ae. atlanticus* – squirrel cycle. SSH virus (which is serologically almost indistinguishable from LAC virus) is associated with an *Ae. canadensis* and *Ae. communis* group – snowshoe hare cycle, whereas LAC virus in the midwest of the United States (and elsewhere) is associated with an *Ae. triseriatus* – chipmunk – tree squirrel cycle (Sudia *et al.* 1971; Pantuwatana *et al.* 1972; Le Duc 1979). California serogroup viruses have been isolated on occasion from alternative arthropods. An example is LAC virus, which has been isolated from *Ae. canadensis* and *Ae. communis* group mosquitoes as well as from tabanids (Karabatsos 1985). SSH virus has been isolated infrequently from *Ae. triseriatus* mosquitoes. In addition, at least six of the California group viruses have been isolated from *Ae. vexans*. The possibility that the viruses may evolve in nature into species that exploit new ecological niches cannot therefore be ignored.




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### 9. Viral determinants of permissive infections of mosquitoes

As all the possible genotype combinations of LAC and SSH virus reassortants are available (Gentsch *et al.* 1977, 1979; Rozhon *et al.* 1981), and because *Ae. triseriatus* mosquitoes are not the normal vectors of SSH virus, the question of the viral determinants for a permissive replication of LAC and SSH virus in that arthropod species has been investigated by employing LAC, or SSH virus, or LAC–SSH reassortant viruses, to infect *Ae. triseriatus* mosquitoes. The results obtained from several studies are summarized in table 2 (Beaty *et al.* 1981*a*, 1982).

Table 2. The role of the bunyavirus M RNA in infection, dissemination and transmission of LAC and SSH parent and SSH–LAC reassortant viruses by *Aedes triseriatus*

| virus L/M/S/ genotype | disseminated infection[a] (%) | transmission[b] (%) |
|---|---|---|
| LAC/LAC/LAC | 100 | 100 |
| SSH/LAC/LAC | 97 | 96 |
| SSH/LAC/SSH | 97 | 90 |
| LAC/LAC/SSH | 12 | 64 |
| SSH/SSH/SSH | 17 | 33 |
| LAC/SSH/LAC | 42 | 42 |
| LAC/SSH/SSH | 8 | 36 |
| SSH/SSH/LAC | 29 | 31 |

[a] Mosquitoes were allowed to engorge on blood–virus mixtures containing either wild-type LAC, SSH, or LAC–SSH reassortants of the indicated L/M/S RNA genotypes. After 14 days of extrinsic incubation the mosquitoes were then analysed. Viral antigen in mosquito tissues was identified by immunofluorescence and the percentage disseminated infections was scored on the basis of the numbers of mosquitoes in which antigen was observed in all tissues, divided by those for which antigen was only located in midgut cells. The LAC/LAC/SSH data used in the dissemination analyses are probably atypical because they involved a reassortant that subsequent analyses (Rozhon *et al.* 1981) demonstrated carried a silent attenuating L defect. In each of the analyses, representing the data from several experiments, between 13 and 45 individual mosquitoes were used (for details see Beaty *et al.* (1982)).

[b] The percentage transmission was scored on the basis of the numbers of individual mosquitoes that transmitted virus and induced disease in the suckling mice (moribund or dead mice) following intrathoracic inoculation of $10^3$ plaque-forming units of virus and after ten days (average) of extrinsic incubation, divided by the number of feeding mosquitoes that exhibited disseminated infections when the engorged mosquitoes were sacrificed and analysed by immunofluorescence. Viruses were inoculated intrathoracically to bypass the mesenteron and thereby preclude variables associated with midgut passage. In each of the analyses, representing the data from several experiments, between 14 and 60 mosquitoes were used (for details see Beaty *et al.* (1981*a*)).

The data have been interpreted as indicating that the LAC viral M RNA gene products (the glycoprotein species) are the principal determinants of the efficiency of LAC virus both to establish a disseminated infection and to be transmitted by *Ae. triseriatus* mosquitoes. By contrast, viruses with an SSH M RNA (SSH glycoproteins) were inefficiently transmitted. Although attenuating mutations in other LAC RNA species may affect the LAC M gene property, as determined with an attenuated LAC/LAC/SSH reassortant virus (L/M/S RNA species, table 3) (Rozhon *et al.* 1981), the major viral determinants of efficient vector transmission appear to be the LAC viral glycoproteins (Beaty *et al.* 1981*a*, 1982).




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TABLE 3. VIRUSES RECOVERED FROM INTRATHORACIC (DUALLY) INFECTED *AEDES TRISERIATUS* MOSQUITOES AND FROM MICE ON WHICH THE INFECTED MOSQUITOES WERE ALLOWED TO FEED[a]

| virus cross | mosquitoes *ts* (%) | mosquitoes *wt* (%) | mice *ts* (%) | mice *wt* (%) |
|---|---|---|---|---|
| SSH I-1 × SSH II-22 | 95 | 5 | 90 | 10 |
| LAC I-20 × LAC II-4 | 45 | 55 | 20 | 80 |
| SSH I-1 × LAC II-5[b] | 35 | 65 | 55 | 45 |
| SSH II-21 × LAC I-20[c] | 100 | 0 | 100 | 0 |

[a] Viruses recovered from dually infected mosquitoes that had been inoculated with *ts* viruses of LAC or SSH virus representing different RNA segments (mutants SSH I-1, SSH II-22, etc.), or viruses obtained from derived moribund and dead mice, were plated on BHK-21 cells at 33 °C, and virus plaques were picked and reassayed at both 33 and 39.8 °C to score for *ts* and wild-type (*wt*) viruses. The results for each cross represent the averages of analyses of several mosquitoes recovered after 7, 14 and 21 days post-inoculation, or of one or more mice obtained after the 7, 14 and 21 day mosquito feedings (for details see Beaty *et al.* 1983).

[b] For different progeny *wt* virus clones, both virus-induced intracellular polypeptides and RNA oligonucleotide fingerprint analyses indicated that the expected SSH/LAC/SSH reassortants (L/M/S RNA species) were present (Gentsch *et al.* 1977; Beaty *et al.* 1981*b*).

[c] The *wt* progeny that would be expected from this cross are rarely obtained even in tissue culture, presumably owing to inefficient gene product interactions of the heterologous viral RNA species (Gentsch *et al.* 1979; Rozhon *et al.* 1981).

## 10. BUNYAVIRUS REASSORTMENT IN MOSQUITOES AFTER INTRATHORACIC INFECTION

From the bunyavirus isolation data it can be concluded that although the viruses are distinct epizootiologically, many are sympatric throughout much of their respective ranges. In theory this would allow ample opportunity for dual virus infections to occur in nature. To investigate this question experimentally, dual infections of laboratory stocks of colonized *Ae. triseriatus* mosquitoes were undertaken with intrathoracic inoculation of *ts* mutants of LAC and SSH viruses. The study yielded evidence for wild-type SSH–LAC reassortant virus formation and for the transmission of the recombinant viruses to a vertebrate host (table 3) (Beaty *et al.* 1981*b*). (In parenthesis, one combination of mutants (SSH II-21 × LAC I-20) did not yield the expected reassortant progeny. Similar results were obtained in tissue culture (Gentsch *et al.* 1977). It was subsequently shown that in tissue culture the expected reassortants are only infrequently obtained (Rozhon *et al.* 1981).) In summary, it has been demonstrated that at least certain intertypic reassortant bunyaviruses can be generated in the arthropod host.

## 11. BUNYAVIRUS REASSORTMENT IN MOSQUITOES AFTER ORAL INFECTION

Studies were done to determine if *Ae. triseriatus* mosquitoes would yield recombinant viruses if they were allowed to acquire the appropriate viruses orally, either simultaneous with or following interrupted feeding (Beaty *et al.* 1985). Mosquitoes were allowed to partly engorge on blood–virus mixtures containing LAC *ts* I-16, LAC *ts* II-5 or wild-type LAC virus (to serve as three control experiments), mixtures of LAC *ts* I-16 and wild-type LAC virus, or LAC *ts* I-16 and LAC *ts* II-5 viruses. As shown in table 4, after 14 days of incubation wild-type viruses were only obtained from the dually infected mosquitoes that fed on blood containing the two *ts* mutants, or from those that received the mutant and wild-type viruses. As expected, only




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TABLE 4. DUAL INFECTION OF *AEDES TRISERIATUS* MOSQUITOES AND GENERATION OF RECOMBINANT VIRUSES[a]

| infection protocol | infection rates 33 °C assay | 40 °C assay |
|---|---|---|
| simultaneous | | |
| LAC *ts* I-16 + LAC *wt* | 15/15 (100%) | 15/15 (100%) |
| LAC *ts* I-16 + LAC *ts* II-5 | 8/8 (100%) | 2/8 (25%) |
| interrupted feeding | | |
| LAC *ts* I-16 then LAC *wt* | 19/19 (100%) | 18/19 (95%) |
| LAC *ts* I-16 then LAC *ts* II-5 | 20/20 (100%) | 4/20 (20%) |

[a] Infection rates are expressed as the number of mosquitoes that were found to contain virus (more than 10 plaque-forming units (p.f.u.)) as detected by plaque assay at 33 or 40 °C, divided by the number tested. Virus–blood meals on which the mosquitoes were initially allowed to feed (partly for the interrupted feeding protocol) contained 6.5–7.3 logs $ml^{-1}$ of each of the indicated viruses. At 2 h post-ingestion the mosquitoes in the interrupted feeding experiment were allowed to engorge to completion on blood–virus mixtures containing 6.5–7.3 logs $ml^{-1}$ of the second virus (LAC *ts* II-5 or LAC wild-type, *wt*, virus). All mosquitoes were held for 14 days, triturated, then assayed for virus. For further details see Beaty *et al.* (1985).

mutant viruses were obtained from the controls that ingested a single *ts* mutant; likewise wild-type virus was recovered from the mosquitoes that only received the wild-type virus inoculum (data not shown). The results of the simultaneous *ts* mutant infections indicated therefore that recombination had occurred in the dually infected mosquitoes.

For the interrupted-feeding studies, mosquities were allowed to partly engorge on blood meals containing LAC *ts* I-16 and two hours later permitted to engorge to completion on meals containing LAC *ts* II-5 (table 4). Wild-type viruses were detected in the mosquitoes after 14 days of incubation. As expected, mosquitoes that received wild-type LAC virus through the interrupted-feeding protocol also yielded wild-type virus (table 4). Thus analyses of the mosquitoes that were superinfected through interrupted-feeding protocol showed that recombinant viruses were produced by this method.

Because bunyavirus RNA segment reassortment would only be epidemiologically significant if the recombinant viruses were transmitted to a vertebrate host, mosquitoes from the above experiment were permitted to feed 14 days after infection on groups of five to seven baby mice. Brains were extracted from the resulting moribund or dead mice and assayed at the permissive (33 °C) and non-permissive (40 °C) temperatures for virus replication. Viruses producing plaques at 40 °C (i.e. with a wild-type phenotype) were isolated from the mice on which mosquitoes that had previously ingested LAC *ts* I-16 followed two hours later by wild-type LAC virus were allowed to feed, indicating that prior infection by the mutant did not preclude transmission of the superinfecting virus. Also, wild-type virus was recovered from the mice that were substrates for feeding by mosquitoes that had been initially infected with LAC *ts* I-16 and then superinfected with LAC *ts* II-5. These results indicated therefore that recombinant viruses could be recovered from a vertebrate host on which a dually infected mosquito had fed.

The preceding experiments indicated a low level of recombinant virus formation (table 4) in both the simultaneous and interrupted feeding protocols. The reason for the low level of recombinants may be trivial (e.g. the particular experimental conditions that were employed), or may be related to the relative numbers of the infecting viruses (etc.). The effects of these and other factors, such as the incubation time, the number of gonadotrophic cycles, or the use of alternative vectors, upon the production of recombinant bunyaviruses have yet to be determined.




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### 12. Bunyavirus interference in mosquito species

In nature, the opportunities for simultaneous infection of vector species involving the ingestion of two or more viruses in a single blood meal are probably severely limited by the acute (short-term) character of bunyavirus infections in a vertebrate host. In most instances, critical viraemia threshold titres for infection of mosquitoes are present for only a few days before the virus is cleared. Infection and production of maximim viraemia in a particular vertebrate host by two viruses simultaneously is probably relatively rare, although it may occur. It can be argued that sequential infection of a vector species is more probable if several blood meals are taken by the female mosquito. It is known that *Ae. triseriatus* females may ingest several blood meals during their lifetime, thereby allowing the possibility for dual virus infection to occur by that route (DeFoliart 1983). Other means of dual virus infections are possible, for instance when an infected male insect inseminates and infects a previously infected female, or when an infected mosquito that has acquired virus transovarially from its mother ingests a blood meal containing an alternate virus.

A series of experiments was therefore done to analyse the potential for bunyavirus superinfection of *Ae. triseriatus* and to determine possible temporal and phylogenetic constraints on these phenomena. In initial experiments, mosquitoes were inoculated intrathoracically with a *ts* mutant of LAC virus (*ts* II-5, an L RNA mutant) and three, seven or fourteen days later superinfected by the same route with SSH *ts* I-1 (an M RNA mutant). After further extrinsic incubation the mosquitoes were triturated in tissue culture medium and the homogenates assayed at 33 and 40 °C to determine the numbers of *ts* and reassortant wild-type viruses respectively. Despite the presence of $10^3$–$10^5$ plaque-forming units of *ts* viruses, no wild-type viruses were detected (Beaty *et al.* 1983). The reverse virus-inoculation schedule (i.e. SSH *ts* I-1 followed by LAC *ts* II-5) gave similar results, suggesting that genetic interaction between the two viruses had been inhibited.

Reassortment can be an event that occurs at a low frequency; therefore, in order to examine the interference phenomemon with a more sensitive procedure, mosquitoes were inoculated intrathoracically with a *ts* mutant of LAC virus and subsequently challenged seven days later by intrathoracic inoculation of either a homologous or heterologous wild-type virus. As wild-type virus progeny can easily be quantified by *in vitro* plaque assays (at 40 °C), the ability of the superinfecting virus to replicate in the mosquito could be determined. The results of the analyses are shown in table 5. They indicated that the mosquitoes were resistant to superinfection with related California group viruses but not to viruses of other families (table 5), or to viruses representing other bunyavirus gene pools (Beaty *et al.* 1983). The data can be interpreted in terms of an interference phenomenon which is specific to viruses that are members of a gene pool.

If mosquitoes became resistant to superinfection by the natural routes of infection, the opportunities for dual infection of vectors and, for bunyaviruses, virus evolution through RNA-segment reassortment, would be limited. In nature, many mosquito species exhibit a behaviour pattern called interrupted feeding. If the defensive reaction of a host causes the mosquito to interrupt its feeding, the vector may complete engorgment at a later time on an alternative host. Thus mosquitoes could ingest blood meals from two different vertebrate hosts which were viraemic with two different viruses in a period of time brief enough to preclude interference. In the light of these considerations, an experiment was conducted to determine when interference to oral superinfection occurs in mosquitoes (Beaty *et al.* 1985).




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Table 5. Parental superinfection of *Aedes triseriatus* mosquitoes previously inoculated with a La Crosse (LAC) *ts* mutant virus[a]

| virus inoculum | | geometric mean titre/log p.f.u. | | |
|---|---|---|---|---|
| day 0 | day 7 | 33 °C assay | 40 °C assay | log difference |
| LAC I-16 | none | 4.5±0.3 | < 1.0 | > 4.5 |
| none | LAC *wt* | 4.5±0.4 | 4.2±0.2 | 0.3 |
| LAC I-16 | LAC *wt* | 4.4±0.1 | < 1.0 | > 3.4 |
| none | SSH *wt* | 3.3±0.5 | 3.0±0.5 | 0.3 |
| LAC I-16 | SSH *wt* | 4.1±0.4 | < 1.0 | > 3.1 |
| none | TAH *wt* | 4.1±0.6 | 3.3±0.5 | 0.8 |
| LAC I-16 | TAH *wt* | 4.1±0.4 | < 1.0 | > 3.1 |
| none | TVT *wt* | 4.6±0.3 | 3.7±0.2 | 0.9 |
| LAC I-16 | TVT *wt* | 3.8±0.2 | < 1.0 | > 2.8 |
| none | WN *wt* | 5.1±0.3 | 4.4±0.2 | 0.7 |
| LAC I-16 | WN *wt* | 5.2±0.3 | 4.4±0.4 | 0.8 |
| none | VSV *wt* | 4.9±0.3 | 4.0±0.4 | 0.9 |
| LAC I-16 | VSV *wt* | 4.5±0.2 | 3.4±0.7 | 1.1 |

[a] Groups of (minimally) four mosquitoes were inoculated intrathoracically with 1.4 log p.f.u. of a LAC *ts* mutant representing the M RNA segment (LAC I-16) and superinfected seven days later by inoculation with 2–4 log p.f.u. of wild-type (*wt*) viruses representing LAC, or other California group bunyaviruses (snowshoe hare, SSH; Tahyna, TAH; trivitattus, TVT), or a flavivirus (West Nile, WN), or a rhabdovirus (vesicular stomatitis virus, VSV). After a further seven days of extrinsic incubation, the presence of *wt* and *ts* viruses in the mosquitoes was determined by plaque assays. For further details see Beaty *et al.* (1983).

Table 6. Interference to LAC virus oral superinfection of *Aedes triseriatus* mosquitoes[a]

| time until ingestion of challenge virus | infection rates 33 °C assay | 40 °C assay |
|---|---|---|
| simultaneous | 15/15 (100%) | 15/15 (100%) |
| 30 min | 8/8 (100%) | 8/8 (100%) |
| 2 h | 19/19 (100%) | 18/19 (95%) |
| 4 h | 7/7 (100%) | 7/7 (100%) |
| 1 d | 18/18 (100%) | 11/18 (60%) |
| 2 d | 11/11 (100%) | 3/11 (27%) |
| 7 d | 6/6 (100%) | 0/6 (0%) |
| 14 d | 5/5 (100%) | 0/5 (0%) |
| 21 d | 3/3 (100%) | 0/3 (0%) |
| 28 d | 4/4 (100%) | 0/4 (0%) |

[a] Infection rates are expressed as the number of mosquitoes that were found to contain virus (more than 10 p.f.u.) as detected by plaque assay at 33 or 40 °C, divided by the number tested. Virus–blood meals on which the mosquitoes were initially allowed to feed (partly) contained 6.5–7.8 log units $ml^{-1}$ of LAC *ts* mutant I-16. At the indicated times post-ingestion the mosquitoes were allowed to engorge to completion on blood–virus mixtures containing 7–7.8 log units $ml^{-1}$ of the challenge wild-type virus. All mosquitoes were held for 14 days after the second meal, triturated, then assayed for virus. For further details see Beaty *et al.* (1985).

*Ae. triseriatus* mosquitoes were permitted to ingest a partial or complete blood meal containing a LAC *ts* mutant virus (LAC I-16). At predetermined times post-feeding, the mosquitoes were then permitted to engorge to repletion on a blood meal containing wild-type LAC virus. One cohort of mosquitoes ingested a meal containing both LAC *ts* I-16 and wild-type virus. As shown in table 6, mosquitoes that received the wild-type virus challenge in the first 24 h replicated the superinfecting virus. Mosquitoes that ingested wild-type virus after

48 h were resistant to superinfection (Beaty *et al.* 1985). Control groups of mosquitoes that received only *ts* virus yielded only *ts* progeny viruses, i.e. the virus was phenotypically stable (Beaty *et al.* 1985). It was concluded from these studies (and the earlier experiment (table 4) demonstrating that reassortment could occur in mosquitoes superinfected two hours after the initial virus infection) that with the greater elapsed time between the two blood meals the mosquitoes became refractory to homologous-virus superinfection.

The molecular basis for the interference phenomenon remains to be determined. Possible mechanisms of interference that require further investigation include the removal of cellular receptors that are used for virus infection of a cell, or development of defective interfering viruses that prevent the replication of a second, genetically interactive, virus. With the available genetic tools further experimentation is required to explore these possibilities and to determine whether interference is operative in transovarially infected mosquitoes.

Whatever the mechanism, the experimental observation of virus interference between genetically permissive viruses, if indeed it has a counterpart in nature, may restrict the ability of a bunyavirus to evolve by RNA-segment reassortment. That reassortment occurs has been shown by analyses of natural virus isolates (Klimas *et al.* 1981*a*; Ushijima *et al.* 1981). However, of some 25 LAC virus isolates that have been analysed, only one intratypic recombinant virus was identified, which may be interpreted to indicate that recombination is a rare event. It is not yet clear whether interference is a viral or a host-mediated phenomenon.

## 13. Virulence characteristics of representative bunyaviruses

Bunyaviruses exhibit a wide range of virulence patterns in vertebrate species. For example, LAC virus is known to cause a severe and sometimes fatal encephalitis in children (Fauvel *et al.* 1980; Thompson *et al.* 1965). Other members of the California group (table 1) such as SSH, Jamestown Canyon, California encephalitis, Inkoo and Tahyna (TAH) viruses, as well as members of certain other bunyavirus serogroups, have also been implicated on occasion in the aetiology of human encephalitis in various regions of the world. Among the group C, Guama, Bwamba, Tataguine and Bunyamwera viruses are agents that are responsible for minor illnesses in humans that almost always involve uncomplicated fevers, or symptoms of fever and rash (Shope 1985). Such viruses are primarily found in the tropics and do not usually cause large outbreaks of human disease. The geographically limited nature of these diseases is probably due to the habitat limits of the normal vector and vertebrate hosts of the individual viruses and the abilities of these hosts to support virus replication. Oropouche virus (Simbu serogroup, table 1) has caused significant outbreaks of human disease in Brazil (Pinheiro *et al.* 1962) involving several thousands of cases both in 1962 and subsequently (Shope 1985).

Among the other members of the family there are viruses that cause haemorrhagic fever (*Nairovirus* genus, Crimean–Congo haemorrhagic fever virus), haemorrhagic fever with renal syndrome (*Hantavirus* genus, Hantaan viruses), sandfly fever and Rift Valley fever (*Phlebotomus* genus). Although Rift Valley fever virus is primarily of significance to the welfare of domestic animals such as sheep and cattle, it is also responsible for severe and sometimes fatal human disease (Imam *et al.* 1978; Meegan 1979). A review of what is known about the pathogenesis, pathology, clinical features and diagnosis of these viruses has been provided recently by Shope (1985).




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## 14. Genetic determinants of the virulence potential of California group bunyaviruses in vertebrates

In model animal systems the virulence characteristics of LAC and TAH viruses differ. Intraperitoneal inoculation of outbred Swiss mice by LAC virus elicits a fatal disease in 4-week-old animals at virus concentrations that do not cause death when TAH virus is employed (Shope *et al.* 1981). SSH virus resembles LAC virus in this regard. By using the available LAC, SSH and TAH reassortant viruses, the genetic basis for these properties has been shown to reside with the bunyavirus M RNA gene products, presumably the viral glycoproteins (table 7). Parental and

Table 7. Intracerebral (i.c.) and intraperitoneal (i.p.) virulence in mice of viruses containing LAC, SSH, or TAH M RNA

| virus | log p.f.u. $ml^{-1}$ | log $LD_{50}$ $ml^{-1}$ i.c. | log $LD_{50}$ $ml^{-1}$ i.p. | index of $LD_{50}$ pfu[b] |
|---|---|---|---|---|
| | viruses with LAC M RNA | | | |
| LAC/LAC/LAC | 9.0 | 7.7 | 4.4 | 1.0 |
| SSH/LAC/SSH | 8.4 | 7.4 | 4.5 | 5.0 |
| SSH/LAC/LAC | 9.0 | 8.3 | 4.0 | 0.4 |
| TAH/LAC/TAH | 8.9 | 7.7 | 4.8 | 3.2 |
| TAH/LAC/LAC | 8.1 | 7.7 | 3.0 | 0.3 |
| LAC/LAC/SSH[c] | 8.2 | 8.3 | 2.5 | 0.1 |
| | viruses with SSH M RNA | | | |
| SSH/SSH/SSH | 8.1 | 8.3 | 4.3 | 6.3 |
| SSH/SSH/LAC | 7.7 | 7.2 | 3.5 | 2.5 |
| LAC/SSH/SSH | 7.4 | 7.7 | 2.8 | 1.0 |
| | viruses with TAH M RNA | | | |
| TAH/TAH/TAH | 9.1 | 9.0 | 0[d] | 0.0013 |
| LAC/TAH/LAC | 7.3 | 7.1 | 0 | 0.063 |
| SSH/TAH/SSH | 7.3 | 6.5 | 0 | 0.063 |
| LAC/TAH/TAH | 8.9 | 8.5 | 0 | 0.002 |
| SSH/TAH/TAH | 8.2 | 7.7 | 0 | 0.01 |

[a] Five 4-week-old mice were inoculated per dilution.
[b] Intraperitoneal (i.p.) 50% lethal dose ($LD_{50}$) p.f.u.$^{-1}$ of La Crosse virus/i.p. $LD_{50}$ p.f.u.$^{-1}$ of virus X.
[c] LAC/LAC/SSH was derived from a cross involving LAC/LAC/LAC *ts* I-20 × SSH/LAC/SSH *ts* II-13 and was found to contain an attenuating L RNA mutation (Rozhon *et al.* 1981)
[d] 0 equals less than 1.6 $LD_{50}$ $ml^{-1}$

reassortant viruses that contain a LAC or SSH M RNA species were found to be more virulent by the intraperitoneal route of inoculation than those with a TAH M RNA species (Shope *et al.* 1981, 1982). One virus that initially appeared to behave aberrantly was a LAC/LAC/SSH reassortant. However, backcross analyses with a distinguishable wild-type LAC virus showed that this particular reassortant contained an attenuating L RNA species. Although it could have been acquired spontaneously, it is likely that the attenuating mutation was induced when a mutagen was used to obtain the LAC *ts* virus from which the reassortant was derived (Rozhon *et al.* 1981). Whatever the origins, the observation demonstrates that in addition to the M RNA species the gene products specified by the other bunyavirus RNA species can affect the viral phenotype.

It has been observed that mice that receive a lethal dose of TAH virus by the intracerebral




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route of inoculation do not live as long as those that receive an equivalent dose of LAC or SSH virus. This is illustrated in table 8, together with data from dose related studies with reassortant LAC, SSH and TAH viruses. Such data again show that the bunyavirus M RNA gene is responsible for this virulence phenotype (Shope *et al.* 1981).

TABLE 8. SURVIVAL OF MICE INOCULATED INTRACEREBRALLY WITH VIRUSES HAVING LAC OR TAH M RNA

| (*a*) small dose (1.0–1.9 log $LD_{50}$)[a] | days ± s.e.m. | | days ± s.e.m. | difference[b] |
|---|---|---|---|---|
| viruses with LAC M RNA | | viruses with TAH M RNA | | |
| LAC/LAC/LAC | 5.4 ± 0.6 | TAH/TAH/TAH | 2.6 ± 0.2 | |
| TAH/LAC/LAC | 6.8 ± 0.4 | LAC/TAH/TAH | 3.6 ± 0.4 | |
| tah/lac/tah | 6.0 ± 1.0 | LAC/TAH/LAC | 2.2 ± 0.4 | |
| SSH/LAC/SSH | 6.8 ± 0.8 | SSH/TAH/SSH | 3.0 ± 0.4 | |
| SSH/LAC/LAC | 3.6 ± 0.2 | SSH/TAH/TAH | 4.6 ± 0.4 | |
| LAC/LAC/SSH[c] | 6.6 ± 0.5 | | | |
| average | 5.8 ± 0.3 | average | 3.2 ± 0.2 | 2.6 |
| (*b*) large dose (3.0–3.9 log $LD_{50}$)[a] | days ± s.e.m. | | days ± s.e.m. | difference[b] |
| viruses with LAC M RNA | | viruses with TAH M RNA | | |
| LAC/LAC/LAC | 3.4 ± 0.2 | TAH/TAH/TAH | 1.8 ± 0.2 | |
| TAH/LAC/LAC | 4.0 ± 0.4 | LAC/TAH/TAH | 2.8 ± 0.4 | |
| TAH/LAC/TAH | 3.9 ± 0.3 | LAC/TAH/LAC | 1.6 ± 0.2 | |
| SSH/LAC/SSH | 4.8 ± 0.4 | SSH/TAH/SSH | 2.2 ± 0.4 | |
| SSH/LAC/LAC | 2.8 ± 0.2 | SSH/TAH/TAH | 3.8 ± 0.4 | |
| LAC/LAC/SSH[c] | 4.4 ± 0.4 | | | |
| average | 3.9 ± 0.2 | average | 2.4 ± 0.2 | 1.5 |

[a] Five 4-week-old mice per dilution. Data are mean ± s.e.m.

[b] Average difference in days between survival of mice receiving viruses having LAC M RNA and mice receiving viruses having TAH M RNA.

[c] LAC/LAC/SSH was derived from a cross involving LAC/LAC/LAC *ts* I-20 × SSH/LAC/SSH *ts* II-13 and was found to have an attenuating L RNA mutation (Rozhon *et al.* 1981).

From the above studies it is clear that the M RNA species of certain California group bunyaviruses, presumably through their gene products, govern the invasion potentials and other virulence characteristics of the viruses. Similar results have been obtained with group C viruses (Bishop *et al.* 1983*a*). Whether these observations extend to other bunyaviruses, or to other members of the Bunyaviridae, remains to be determined. Apart from such overall characteristics the results say nothing about the tissue tropisms of the individual viruses, nor why LAC but not TAH virus is lethal after intraperitoneal inoculation, nor why TAH virus kills mice faster than LAC virus after intracerebral inoculation.

In a study designed to follow the infection course of California group bunyaviruses following subcutaneous inoculation in mice, Tignor *et al.* (1983) have reported that there is an antigenaemia (viraemia) that extends from 18 to 40 h after inoculation. Viral antigen was observed in the lumina of capillaries and in phagocytic cells adjacent to these vessels. For some viruses, antigen was also observed in chondrocytes as well as fibroblasts and basement membranes adjoining connective tissues. LAC virus antigen has been observed in muscle fibre cells and at sites that appeared to represent neuromuscular junctions, raising the possibility




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that such sites may have a role to play in the entrance of the virus into the central nervous system. Such sites did not seem to be infected by TAH or LAC/TAH/LAC reassortant viruses (Tignor *et al.* 1983).

Distinct histopathological lesions in the brains of mice inoculated intracerebrally with various California serogroup viruses have been identified for LAC, TAH and trivittatus viruses (Shope *et al.* 1982). LAC virus induced a lytic, mildly inflammatory encephalitis with marked necrosis in the olfactory cortex and in the pyramidal cells of the hippocampus. TAH virus infection was also characterized by lesions in these tissues, but in addition there was a striking focal necrosis in the granular layer of the cerebellum. In most mice inoculated intracerebrally with TAH virus, by the time of death the amount of perivascular inflammation was not as marked as that observed for the LAC-virus-infected brains. No lesions were identified in the brains of mice that received TAH virus by the intraperitoneal route of inoculation. Mice infected intracerebrally with trivittatus virus survived longer than those infected with LAC or TAH virus. It was observed that for trivittatus-infected brains there was a loss of neurons, as well as gliosis and demyelinating signs in the olfactory region. In addition there was focal necrosis in the granular layer of the cerebellum and a marked inflammatory response throughout the brain. One lesion that appeared to be specific to trivittatus virus infections was neurolysis in the dendate gyrus.

## 15. Conclusions

Analyses of the biological, molecular and genetic attributes of California group members of the Bunyaviridae have contributed significantly to what is known about the infection potentials of arboviruses both in the vertebrate host and in potential arthropod vectors. The two host systems represent considerably different environments for virus infections. In the vertebrate the virus has to elicit, within a short period of time, a sufficient viraemia for transmission to a blood-sucking arthropod to occur. The incidence of diseases such as encephalitis may be incidental to the production of a viraemia, or it may predispose the infected animal to becoming an easier target for the arthropod vector. In the arthropod host the virus has to replicate in various tissues in order to be transmitted horizontally (to a vertebrate) or vertically (to an offspring). The availability of genetic and molecular tools with which to address the questions that pertain to each of these situations has allowed considerable progress to be made in understanding virus–vector–host relations.

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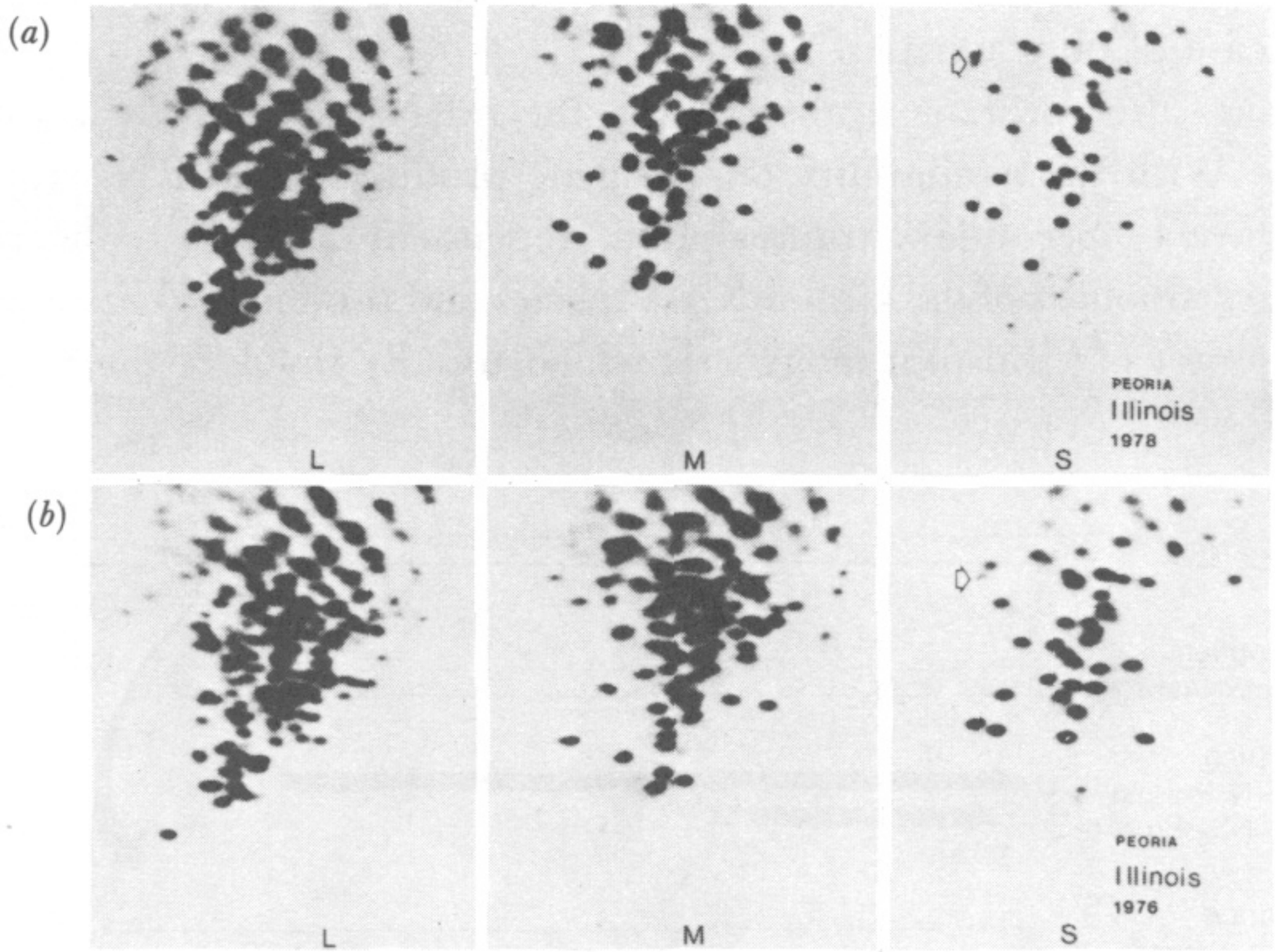


FIGURE 5. Oligonucletde fingerprint analyses of two La Crosse virus isolates obtained from mosquitoes collected at the same site in Peoria, Illinois, but at different times. (*a*) 1978; (*b*) 1976. (See Klimas *et al.* (1981 *a*) for further examples.)

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