Bunyavirus superinfection and segment reassortment in transovarially infected mosquitoes

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Rapid evolution of bunyaviruses may occur by RNA segment reassortment between closely related viruses. Reassortment between viruses occurs in dually infected mosquitoes when two different viruses are simultaneously ingested or when the second virus is ingested within 2 days of the first virus. By 3 days after oral infection, interference to superinfection occurs, thus limiting the potential for evolution. *Aedes triseriatus* mosquitoes can also be transovarially infected (TI) with LaCrosse (LAC) virus. In these studies, the potential for oral superinfection of TI mosquitoes was assessed. Approximately 20% of mosquitoes TI with either a temperature-sensitive LAC virus or wild-type (wt) LAC virus became superinfected by ingesting blood meals containing wt LAC or snowshoe hare (SSH) viruses. LAC virus TI mosquitoes superinfected with SSH virus were detected by blot hybridization or RT–PCR. Viruses from these mosquitoes were plaque purified and genotyped using RT–PCR. Reassortant genomes were detected in 2–3% of the viruses genotyped, and 4–0% of the genomes tested were diploid for one genome segment.

Introduction

The California serogroup bunyaviruses can undergo rapid evolution via gene segment reassortment (antigenic shift) in mosquitoes that become infected orally with two different viruses (Beaty et al., 1985). Reassortment of heterologous as well as homologous viruses can occur in mosquitoes, followed by oral and transovarial transmission of reassortant genotypes (Chandler et al., 1990, 1991; Turell et al., 1990). In nature, mosquitoes probably only rarely ingest two viruses simultaneously. It is more likely that they become infected with one virus and are exposed to a second virus at a later time. However, *Aedes triseriatus* mosquitoes that have been orally infected with LaCrosse (LAC) virus become refractory to oral superinfection with a second virus over a short period of time (Beaty et al., 1985; Sundin & Beaty, 1988). This interference phenomenon correlates with virus replication in the midgut following an infective blood meal. The level of interference increases over time from 24 h to 7 days, and is proportional to virus titre in the midgut (Sundin et al., 1987). Thus, interference may suppress bunyavirus evolution in nature by preventing mosquitoes from being superinfected with a second virus.

LAC virus is transovarially transmitted by *A. triseriatus* efficiently (Beaty & Bishop, 1988). Transovarially infected (TI+) mosquitoes may subsequently ingest a viraemic blood meal. If interference to superinfection also occurs in TI+ mosquitoes, virus evolution via gene segment reassortment would be restricted. Because virus titres in all tissues of TI+ mosquitoes are lower than in orally infected mosquitoes (Turell et al., 1982), we hypothesized that TI+ mosquitoes could be superinfected with a second virus when exposed to a viraemic blood meal.

In this study, we demonstrate that TI+ mosquitoes do not exhibit complete interference to superinfection. Multiple approaches were utilized to demonstrate that a significant proportion of TI+ mosquitoes can become superinfected, and that segment reassortment can occur in dually infected mosquitoes.

Methods

- **Viruses.** Two wild-type (non-temperature-sensitive) California serogroup bunyaviruses, LAC and snowshoe hare (SSH) viruses, were used to transovarially infect and/or orally challenge mosquitoes. The
Table 1. Primers and amplification products in RT–PCR assays

<table>
<thead>
<tr>
<th>Virus genome segment</th>
<th>Product size (bp)</th>
<th>VS primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAC</td>
<td>517</td>
<td>TCAGGCTCTTGGCAATGGCCGTC</td>
</tr>
<tr>
<td>SSH</td>
<td>393</td>
<td>AATTTAGAACCTAATTTGAATG</td>
</tr>
</tbody>
</table>

Prototype viruses were obtained from the Yale Arbovirus Research Unit, New Haven, CT, USA. A temperature-sensitive (ts) LAC virus mutant, L-1-16, which contained a ts lesion in the M RNA segment, was obtained from D. H. L. Bishop, University of Alabama, Birmingham, AL, USA. The origin and passage history of the viruses used have been described previously (Beaty et al., 1985). To prepare virus stocks, BHK-21 cells were inoculated with LAC virus at an m.o.i. of 0.01. When cells exhibited CPE, the culture medium was centrifuged to remove cells, and the supernatant was diluted to 20% in foetal bovine serum, divided into aliquots and stored at $-70^\circ$C.

Mosquitoes. *A. triseriatus* mosquitoes were from a colony that originated from mosquitoes collected in La Crosse, WI, USA. This colony has been maintained at AIDL continuously since 1982. Mosquitoes were reared at 23 °C, 80% relative humidity with a photocycle of 16 h light:8 h dark. Mosquitoes were provided sugar cubes, raisins and water ad libitum.

Establishment of T+ *A. triseriatus* colonies. The wt LAC and L-I-16 viruses were used to establish colonies of T+ *A. triseriatus* mosquitoes. First generation mosquitoes were infected with a ts LAC virus by intrathoracic inoculation or with wt LAC virus orally. T+ mosquitoes were used to found the next generation. Adult mosquitoes were screened for virus infection by fluorescent antibody (FA) testing. One leg was removed, squashed onto a microscope slide and assayed by FA (Beaty et al., 1985). T+ mosquitoes were used in these superinfection studies.

Oral challenge of mosquitoes. T+ and control mosquitoes were orally challenged with virus within 2 weeks of eclosion. T+ mosquitoes ingested either wt LAC or SSH virus via infected blood meal, and then were held for 2 weeks extrinsic incubation (EI). Control mosquitoes (T−) ingested the same virus-containing blood meal. After 2 weeks EI, mosquitoes were assayed for superinfecting virus.

Quantification of virus in mosquito midguts. Midguts of T+ mosquitoes were individually tested by plaque assay in Vero cells to determine the virus titre. Midguts of orally infected mosquitoes were assayed at 1, 2, 3 and 7 days post blood feeding for infectious virus by plaque assay in Vero cells.

Assay for superinfection. Mosquitoes were assayed for the presence of superinfecting virus by three different techniques: (1) biological assay for infectious virus at a non-permissive temperature; (2) molecular hybridization; and (3) RT–PCR using primers designed to distinguish LAC virus RNA segments from SSH virus RNA segments.

(1) Biological assay. Mosquitoes that were T+ with ts LAC virus and orally challenged with wt LAC virus were assayed for wt LAC virus by comparison of titres obtained by plaque assay in Vero cells at permissive (33 °C) and non-permissive (40 °C) temperatures (Beaty et al., 1985).

(2) Hybridization assay. Mosquitoes that were T+ with wt LAC virus and orally challenged with SSH virus were assayed for SSH virus by nucleic acid hybridization (Chandler et al., 1990). Tritrated mosquitoes were subjected to plaque assay in Vero cells. Well-isolated plaques were identified and picked individually. Virus from each plaque was amplified in BHK-21 cells and total cytoplasmic RNA was extracted from the cells (White & Bancroft, 1982). The RNA was blotted onto Nytran membranes and subjected to hybridization with a cDNA probe specific for the LAC virus L RNA segment (pLAC 4.16; Chandler et al., 1990, 1991). Blots were prehybridized for 6 h at 46 °C in a solution containing 5 x SSC, 5 x Denhardt’s solution, 20 mM...
µ0 to either LAC virus RNA or to SSH virus RNA (Table 1). The RT–PCR bound to both LAC and SSH virus RNA and the VS primer hybridized performed as described above. In each primer set, the VC primer was using an RNAgents kit (Promega). The RT–PCR reactions were monolayers of BHK-21 cells. Virus was allowed to replicate until CPE was days after infection and inoculated into 24-well plates containing (1992) were used to genotype viruses generated by plaque isolation.

(3) RT–PCR assay. Mosquitoes that were TI+ with wt LAC virus and orally infected with SSH virus were assayed for the presence of both wt LAC virus and SSH virus S segment RNA using RT–PCR. Each mosquito was triturated in 500 µl MEM. Approximately 250 µl homogenate was removed and stored at –70 °C for use in plaque isolation of viruses. The remaining 250 µl was centrifuged, the supernatant was removed and 200 µl denaturation solution (containing guanidine thiocyanate and 2-mercaptoethanol) was added. RNA was extracted using 20 µl 2 M sodium acetate (pH 4.0) and 200 µl phenol:chloroform:isoamyl alcohol solution), precipitated, washed and resuspended in 20 µl RNase-free water. For synthesis of LAC or SSH virus S segment cDNA, total cellular RNA [approximately 0.5 µg RNA in 5 µl nuclease-free H2O (Promega)] was mixed with 15 pmol virus-complementary (VC; Table 1) primer in 7.7 µl nuclease-free water. Samples were denatured at 70 °C for 10 min, then cooled to 20 °C for primer annealing. RNA was reverse transcribed in a 20 µl buffer solution containing 10 mM of each dNTP and 60 U SuperScript II reverse transcriptase (Gibco-BRL) at 42 °C for 1 h, then stopped at 95 °C for 10 min. For PCR, 2 µl cDNA was added to a 50 µl reaction mixture containing 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 0.1% Triton X-100, 200 mM each dNTP, 1.5 U Taq DNA polymerase (Promega) and 50 pmol each VC and virus-sense (VS) primers (Table 1). The sample was subjected to PCR under the following conditions: 92 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min for 30 cycles, followed by a final extension at 72 °C for 10 min. PCR products were analysed by electrophoresis in 2% agarose gels. Stored homogenates of mosquitoes shown by RT–PCR to be dually infected were subjected to plaque isolation of viruses.

Genotyping of reassortant viruses by RT–PCR. PCR primers designed to distinguish LAC virus L, M and S RNA segments from analogous SSH virus RNA segments designed by Urquiñi & Bishop (1992) were used to genotype viruses generated by plaque isolation. Approximately 24 plaques per superinfected mosquito were picked 6 days after infection and inoculated into 24-well plates containing monolayers of BHK-21 cells. Virus was allowed to replicate until CPE was observed (24–48 h), at which time RNA was extracted from the cells using an RNA agents kit (Promega). The RT–PCR reactions were performed as described above. In each primer set, the VC primer was bound to both LAC and SSH virus RNA and the VS primer hybridized to either LAC virus RNA or to SSH virus RNA (Table 1). The RT–PCR products generated were of distinct sizes so the parental origin of the segment could be easily identified (Table 1).

Results

Oral infection of mosquitoes

The oral infection rates of control mosquitoes with LAC or SSH virus did not differ statistically. TI− mosquitoes were susceptible to oral infection with both wt LAC virus and SSH virus: 95.5% (85/89) of the mosquitoes became infected with LAC virus and 81.7% (89/109) became infected with SSH virus.

Quantification of virus in midguts

To determine if a lower virus titre was present in the midguts of TI+ mosquitoes than in orally infected mosquitoes, virus titre was quantified in the midguts of TI+ mosquitoes and in the midguts of TI− mosquitoes that had been orally infected with LAC virus. TI+ mosquitoes contained low virus titres in their midguts. Titres in nine mosquitoes ranged from 3.2–103 to 3.2–105 p.f.u. per midgut and averaged 1.4×102 p.f.u. per midgut. Midgut titres of mosquitoes that had been fed virus were dramatically higher, ranging from 3.4–103 p.f.u. per midgut at 24 h after feeding to 5.4–104 p.f.u. per midgut at 7 days after feeding (Sundin & Beaty, 1988).

Oral superinfection of TI+ mosquitoes

TI+ mosquitoes were susceptible to superinfection with homologous and heterologous viruses. When mosquitoes that were TI+ with wt LAC virus were orally challenged with wt LAC virus, 18.6% became superinfected (Table 2). Mosquitoes TI+ with wt LAC virus and challenged with SSH virus were also superinfected (19.6%; Tables 1 and 2) as determined by blot hybridization (Fig. 1). The proportion of plaque-purified viruses containing the SSH virus L RNA ranged from 4% to 29% per mosquito (Table 3). When mosquitoes that were TI+ with wt LAC virus were orally challenged with SSH virus, 19.5% became superinfected as determined by RT–PCR (Tables 2 and 4).

Generation of reassortant viruses in dually infected mosquitoes

RT–PCR was used to determine if genome segment reassortment had occurred in dually infected mosquitoes. Viruses from 12 wt LAC virus TI+ mosquitoes identified as being orally superinfected with wt SSH virus were plaque-purified and genotyped (Table 4). Of 255 viruses analysed, a complete genotype was obtained for 195 viruses. Of the viruses genotyped, 92.8% were LAC virus parental genotype (181/195), 1.5% were SSH virus parental genotype (3/195), 2.1% had reassortant genotypes (4/195) and 3.6% had diploid genotypes (7/195). Two of the reassortant viruses derived the L segment from the SSH virus parent and the M and S segments from the LAC virus parent (genotype SSH/LAC/LAC); Table 4). One reassortant virus derived the L and M segments from the LAC virus parent and the S segment from the SSH virus parent (genotype LAC/LAC/SSH). The remaining reassortant acquired the L and S segments from the LAC virus parent and the M segment from the SSH virus parent (genotype LAC/SSH/LAC).

Seven viruses that were genotyped were diploid for one of the three segments (Table 4). All of the diploid viruses had LAC virus L, M and S segments and one additional segment from the SSH virus parent. The S segment was the most
Table 2. Superinfection of transovarially infected *A. triseriatus* mosquitoes with homologous and heterologous virus

*ts LAC* virus TI+ mosquitoes were orally challenged with homologous or heterologous virus. Three different techniques were used to detect superinfected mosquitoes.

<table>
<thead>
<tr>
<th>Transovarially infecting virus</th>
<th>Challenge virus (titre)</th>
<th>Method of detecting superinfection</th>
<th>Mosquitoes superinfected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ts LAC</em></td>
<td>wt LAC (7.5*)</td>
<td>Growth at 40 °C</td>
<td>18.6% (8/43)</td>
</tr>
<tr>
<td><em>ts LAC</em></td>
<td>SSH (7.8*)</td>
<td>Hybridization</td>
<td>19.6% (11/56)</td>
</tr>
<tr>
<td>wt LAC</td>
<td>SSH (5.5†)</td>
<td>RT–PCR</td>
<td>19.5% (17/87)</td>
</tr>
</tbody>
</table>

* Titre is expressed as *log*10 p.f.u. per ml.
† Titre is expressed as *log*10 TCID50 per ml.

Fig. 1. Detection of superinfection by blot hybridization. (A) Twenty-four plaques from each mosquito orally infected with either LAC or SSH virus; hybridized with the LAC virus L RNA probe. (B) Twenty-four plaques from a mosquito transovarially infected with *ts LAC* virus and orally challenged with SSH virus. Plaque 4 is SSH virus genotype.

common diploid segment (71%) (Table 4, Fig. 2). Two other diploid viruses with incomplete genotypes were also identified; one was diploid for the S segment and the other was diploid for the L segment. These two diploid viruses were not included when calculating the percentage of diploid viruses due to their incomplete genotypes.

Sixty plaque-purified viruses could not be completely genotyped. The S segment was most commonly missing (63.3% of plaques had no S segment detectable) and the L segment was most readily detected (26.7% of plaques had no L segment detectable).

Table 3. Superinfection of *ts LAC* virus TI+ mosquitoes orally challenged with SSH virus, based on blot hybridization with L RNA probe

<table>
<thead>
<tr>
<th>Parental origin of L RNA segment</th>
<th>Mosquito no.</th>
<th>LAC* (%)</th>
<th>SSH* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>23 (92)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24 (96)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>17 (68)</td>
<td>7 (29)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>20 (80)</td>
<td>4 (16)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>21 (84)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>22 (88)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>23 (92)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>23 (92)</td>
<td>1 (4)</td>
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</tr>
<tr>
<td>32</td>
<td>17 (68)</td>
<td>7 (29)</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>20 (80)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>23 (92)</td>
<td>1 (4)</td>
<td></td>
</tr>
</tbody>
</table>

* Number of plaques from each mosquito that were genotyped as LAC or SSH viruses by blot hybridization. The numbers in parentheses indicate the percentage of plaques derived from each parent virus.

SSH virus from infected mosquitoes tended to produce smaller plaques than the LAC virus-infected controls. Therefore, plaques from three mosquitoes (numbers 163, 135 and 129) were tested to determine if plaque size correlated with genotype. Each plaque was numbered and the size of the plaque was recorded as large (approximately 4 mm or greater), medium (2–3.5 mm), small (approximately 1–2 mm) or very small (1 mm or less). The plaque size did not appear to correlate with the genotype (data not shown). Viruses that were genotyped as having all LAC virus segments produced plaques of all sizes. Two reassortant viruses (163-20 and 163-22) that
Table 4. SSH virus superinfection of LAC virus TI+ mosquitoes and reassortment of the two viruses

wt LAC virus TI+ mosquitoes were superinfected orally with SSH virus. Superinfected mosquitoes were detected via RT–PCR and plaque-purified viruses were genotyped. RT–PCR was used to determine the number of parental, reassortant, and diploid genotypes present.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquitoes superinfected</td>
<td>17/87 (19.5%)</td>
</tr>
<tr>
<td>Superinfected mosquitoes plaque assayed</td>
<td></td>
</tr>
<tr>
<td>Plaque-purified viruses genotyped</td>
<td>12</td>
</tr>
<tr>
<td>Viruses with complete genomes</td>
<td>255</td>
</tr>
<tr>
<td>LAC virus parental genotypes</td>
<td>195</td>
</tr>
<tr>
<td>SSH virus parental genotypes</td>
<td>3/195 (1.5%)</td>
</tr>
<tr>
<td>Reassortant genotypes of four plaque-purified viruses:</td>
<td></td>
</tr>
<tr>
<td>SSH/LAC/LAC*</td>
<td>4/195 (2.1%)</td>
</tr>
<tr>
<td>LAC/LAC/SSH*</td>
<td>1</td>
</tr>
<tr>
<td>LAC/SSH/LAC*</td>
<td>1</td>
</tr>
<tr>
<td>Diploid genotypes:</td>
<td></td>
</tr>
<tr>
<td>Diploid L segment</td>
<td>7/195 (3.6%)</td>
</tr>
<tr>
<td>Diploid M segment</td>
<td>1</td>
</tr>
<tr>
<td>Diploid S segment</td>
<td>5</td>
</tr>
</tbody>
</table>

* Parental origin of L, M, and S segments, respectively.

Fig. 2. RT–PCR products of viruses diploid for the S segment. Viral RNA was RT–PCR amplified using primers specific for the S segment of LAC virus (lanes A, C, E and G) and with primers specific for the SSH virus S segment (lanes B, D, F and G). Lanes: A and B, the products generated from LAC and SSH virus control RNA (respectively); C and D, RT–PCR products amplified from mosquito 127, virus 7 RNA (LAC and SSH primers, respectively); E and F, RT–PCR products amplified from mosquito 155, virus 5 RNA (LAC and SSH primers, respectively); G, negative control.

had the same genotype (SSH/LAC/LAC) had different sized plaques: medium and very small. No parental SSH virus genotypes were detected in the experiment to correlate plaque size and genotype.

Discussion

The results clearly demonstrate that a portion of LAC virus TI+ mosquitoes are susceptible to oral superinfection with related viruses. This greatly enhances the possibility for reassortment to occur in nature. For example, LAC virus is efficiently transovarially transmitted by and overwinters in A. triseriatus mosquitoes (Beaty & Bishop, 1988). As demonstrated in these studies, a significant proportion of transovarially infected mosquitoes are susceptible to superinfection (in contrast to orally infected mosquitoes), which could result in segment reassortment and the evolution of new virus genotypes. Such a mechanism could greatly increase the evolutionary potential of bunyaviruses.

The ability of TI+ mosquitoes to become superinfected with homologous or heterologous virus was demonstrated by assays using temperature sensitivity, blot hybridization and virus-specific RT–PCR. All three techniques demonstrated that approximately 20% of the TI+ mosquitoes became superinfected when orally challenged. The biological ts assay was used to determine if superinfection had occurred. The RT–PCR assay allowed us also to determine if reassortment had occurred, and allowed reassortant viruses to be completely genotyped. Four reassortant viruses were detected using RT–PCR (Table 4). Interestingly, three of these four reassortant viruses had LAC virus M segments. LAC–SSH reassortant viruses that have the LAC virus M segment are much more efficient at dissemination in and transmission by A. triseriatus mosquitoes (Beaty et al., 1981, 1982; Schopen et al., 1991). Other important phenotypic characteristics also segregate with the M segment, including neuroinvasiveness (Beaty et al., 1981; Gonzalez-Scarano et al., 1992).

LAC virus and LAC virus RNA segments were over-represented in the analyses. Two of the three segments of each reassortant virus were LAC virus segments and the LAC virus
parental genotype was present in 92.8% of the plaques tested. This may be due to the fact that the mosquitoes were TI+ with LAC virus, which may have limited the replication sites for SSH virus. In addition, *A. triseriatus* mosquitoes are the natural host of LAC virus but not SSH virus, potentially providing a replicative advantage to the former virus.

Several studies have documented the presence of diploid viruses belonging to the family *Bunyaviridae* (Rozhon et al., 1981; Iroegbu & Pringle, 1981; Hampson, 1987; Urquidi & Bishop, 1992; Rodriguez et al., 1998). Bunyavirus virions are pleomorphic (Talmon et al., 1987) and this may allow the packaging of additional nucleocapsid segments (Urquidi & Bishop, 1992). The techniques used to genotype viruses (e.g., RT–PCR, blot hybridization, ts mutants) would not detect viruses that are diploid or polyploid for segments of the same parent. Thus, polyploid viruses may be relatively common (Rodriguez et al., 1998). The S segment was the segment that was most commonly heterodiploid (Table 4). Urquidi & Bishop (1992) also found that a majority of LAC–SSH reassortant diploid viruses contained two S segments, which are the most abundant intracellular RNA species (Gentsch et al., 1977; Bishop & Shope, 1979). Their abundance and small size may make it structurally more feasible for the virus to package two or more of them.

It is possible that the diploid viruses detected may have actually been due to the presence of two overlapping plaques. However, only plaques that were well separated were picked to be genotyped and, in each case, only one of the viral segments was found to be diploid. If the diploidy were due to overlapping plaques, one would expect to detect all three segments of both parental viruses.

The S segment primers identified superinfected mosquitoes more effectively than the L or M primer sets did. Sixty-nine of the mosquitoes initially screened for superinfection using the S segment primers were later screened with the L and M segment primers. When screened with the S segment primers, 21.7% (15/69) of these mosquitoes were identified as superinfected. When the M segment primers were used, 10.1% (7/69) mosquitoes were identified as superinfected. When the L segment primers were used, 18.8% (13/69) were superinfected. These data indicate that the M segment primers are not as sensitive as the other two primer sets in detecting dual infections. Therefore, it is possible that some M segment diploid viruses were not detected by the RT–PCR technique. In this regard, passage of reassortant bunyaviruses may result in loss of the heterologous segment (Rodriguez et al., 1998). Therefore, passage of plaques to BHK cells for further amplification before genetic analysis may have resulted in the underestimation of the number of diploid viruses initially produced.

These studies have demonstrated that interference to superinfection is not complete in TI+ mosquitoes. TI+ mosquitoes have dramatically lower titres of virus present in their midguts than orally infected mosquitoes, which may permit them to become superinfected when challenged with a second virus via blood feeding. It is interesting to note that while SSH virus overall infects a smaller proportion of previously non-infected mosquitoes (82% vs 96% for LAC virus), the superinfection rate in TI+ mosquitoes was equivalent to that of LAC virus. This suggests that there may be less interference to a heterologous superinfecting virus than to a homologous virus in infected mosquitoes, thereby facilitating dual infection and RNA segment reassortment.

The frequency of reassortment following superinfection of TI+ mosquitoes was relatively low compared to rates obtained when vectors were infected simultaneously or via interrupted feeding (Beaty et al., 1985). It is noteworthy in this regard that virus replication and reassortment is enhanced in the ovaries of female *A. triseriatus* that are provided with additional blood meals. Active transport of molecules into and rapid cell division in the ovarian follicles may overcome interference (Chandler et al., 1990). The mosquitoes in this study received only one blood meal and the ovaries were not metabolically active at the time of superinfection. An additional blood meal may have increased the generation of reassortant viruses. Nonetheless, since transovarial transmission is a major maintenance and amplification mechanism for LAC virus in nature (Beaty & Thompson, 1975; Watts et al., 1974), the ability of even a small percentage of TI+ mosquitoes to become superinfected and to generate reassortant viruses may be epidemiologically significant.

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References


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