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GENETIC RELATEDNESS OF THE KEMEROVO SEROGROUP VIRUSES: II. RNA-RNA BLOT HYBRIDIZATION AND GENE REASSORTMENT *IN VITRO* OF THE GREAT ISLAND SEROCOMPLEX

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Summary. — The majority of the thirty-two Great Island serocomplex isolates examined exhibit distinct dsRNA polyacrylamide gel profiles. Yet, these viruses are closely related by blot hybridization with only two genes showing significant sequence divergence. Gene reassortment was demonstrated between selected pairs of the Great Island serocomplex viruses with two different geographic regions represented. The majority of the reassortant progeny from the cross of selected pairs resulted in progeny with multiple gene-replacements. The ability of these selected isolates to reassort confirms the close taxonomic relationship of the isolates in spite of their geographic distribution.

Key words: *blot hybridization; dsRNA; genetic relatedness; Great Island serocomplex; Orbivirus; reassortment*

Introduction

Kemerovo serogroup viruses are associated with argasid and ixodid ticks. The Kemerovo serogroup was divided into four serocomplexes based on different levels of cross-reactivity in complement-fixation tests (Casals, 1971; Main *et al.*, 1976). The four serocomplexes are Chenuda, Cape Wrath-Great Island, Kemerovo, and Seletar-Wad Medani. The antigenic division of the isolates into serocomplexes appears to coincide with the geographic distribution of the isolates and with the species of ticks which vector the different isolates. The Great Island serocomplex viruses are maintained in nature by hard-bodied ticks, *Ixodes uriae*, which are associated with sea bird colonies found in the polar and subpolar regions of both hemispheres. At least, twenty serotypes within the Great Island serocomplex are recognized currently.

Serologic tests assess the relatedness of genes which encode viral antigenic determinants, and thus, serologic estimates are based on the sequence con-

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servation of one or two genes. Blot hybridization and gene reassortment have been used to assess the sequence and functional relatedness of members of *Orbivirus* serogroups (Bodkin and Knudson, 1985*b*, 1986, 1987; Gonzalez and Knudson, 1987*a*, 1987*b*, 1988; Brown *et al.*, 1988*a*, 1988*b*; Kowalik and Li, 1987). The relatedness of members of the Kemerovo serocomplex were examined by blot hybridization and gene reassortment (Brown *et al.*, 1988*b*). While the results confirmed the serologic findings which suggest that the Kemerovo serocomplex contains two virus types, blot hybridization demonstrated that all ten genes were highly conserved among members of the same type, but genes were not as highly conserved between types. Although each virus type had a different geographic distribution and was vectored by a different tick species, gene reassortment results indicated that the two virus types belonged to the same gene pool. In contrast, the majority of the Great Island serotypes have a different geographic distribution, and they are vectored by the same tick species.

In this tudy, RNA-RNA blot hybridization was used to assess the genetic relatedness of the Great Island serotypes. The serotypes examined were Bauline, Cape Wrath, Fin Isolates, Great Island, Mykines, St. Abb's, and Tindholmur from the North Atlantic; Kenai, Okhotskiy, Poovot, and Yaquina Head from the North Pacific; and Nugget from the South Pacific (Main *et al.*, 1973, 1976; Lvov *et al.*, 1973; Yunker *et al.*, 1973; Ritter and Feltz, 1974; Doherty *et al.*, 1975; Yunker, 1975; Main, 1978; Saiku *et al.*, 1980; Nuttall *et al.*, 1981). Gene reassortment among selected isolates from two different geographic areas in the North Atlantic was used to examine the functional relatedness of the Great Island isolates. The hybridization and reassortment data are discussed with respect to the antigenic relationships, geographic distribution, and the tick host of the Great Island serocomplex viruses.

Materials and Methods

Virus stocks and tissue culture. Great Island serocomplex viruses and the virus designations used in this study are listed in Table 1. Viruses were grown in BHK-21 cells as described previously (Gonzalez and Knudson, 1987*a*).

Plaque assay and reassortment in vitro. A plaque assay procedure utilizing an agarose-nutrient overlay was used to titrate virus stocks and reassortant viruses (Buckley, 1974; Gonzalez and Knudson, 1987*b*). Briefly, viral suspensions were prepared which consisted of the two parental virus stocks in varying multiplicity of infection (M.O.I.) ratios. BHK-21 cells grown in microtest plates (96-well) were infected with the viral suspensions. The infected plate was incubated at 32 °C for 24 hr. The infected cells were harvested and frozen at − 70 °C. On the day of the reassortment experiment, a plaque assay titration of the parental viruses was used to calculate the M.O.I. for each mixed virus ratio. The infected cell harvests were plated in plaque assays, plaques were picked, and the plaques were prepared for the infection of cells grown in a 24-well cluster plate. Viral dsRNA was extracted from the infected cells, electrophoresed in a polyacrylamide gel and stained with ethidium bromide. The dsRNA profiles were scored for the parental origin of the segments. Viral dsRNA of selected reassortants was 3′ end-labeled and electrophoresed in a polyacrylamide gel.

Polyacrylamide electrophoresis (PAGE). Viral dsRNA was isolated from infected BHK-21 cells which were grown in 24-well cluster plates (Travassos da Rosa *et al.*, 1984). Polyacrylamide gels used for electrophoretic transfer of dsRNA were electrophoresed at 20 mAmps for either 20 or 30 hr. The dsRNA of selected reassortants were labeled and electrophoresed for 20 hr at 10 mAmps in a polyacrylamide gel (Gonzalez and Knudson, 1987*b*).

Table 1. Great Island serocomplex virus isolates

Virus isolate	Designation	Source	Microhabitat origin	Geographical	Year[a]	Profile[b]
Bauline (BAU) CanAr 14	BAU	*Ixodes uriae* 10 engorged nymphs	Puffin colony (*Fratecula artica*)	Great Island Newfoundland Canada	23 July 71	
Bauline (BSU) CanAr 63	BAU 63	*Ixodes uriae* 6 engorged males	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	12 July 72	
Bauline (BAU) CanAr 128	BAU 128	*Ixodes uriae* 11 engorged females	Herring gull (*L. argentatus*)	Great Island Newfoundland Canada	20 July 72	
Bauline (BAU) CanAr 133	BAU 133	*Ixodes uriae* 9 engorged nymphs	Herring gull (*L. argentatus*)	Great Island Newfoundland Canada	20 July 72	BAU 128
Bauline (BAU) CanAr 172	BAU 172	*Ixodes uriae* 10 engorged females	Herring gull (*L. argentatus*)	Great Island Newfoundland Canada	31 July 72	
Cape Wrath (CW) ScotAr 20	CW 20	*Ixodes uriae* 1 engorged female	Common murre colony (*Uria aalge*)	Clo Mor Cape Wrath Scotland	June 73	
Fin isolates (FI) Fin NorV-808	FI 808	*Ixodes uriae*		Lofoten, Norway Rost Islands	5—6 July 74	
Fin isolates (FI) Fin NorV-873	FI 873	*Ixodes uriae*		Lofoten, Norway Rost Islands	5—6 July 74	
Fin isolates (FI) Fin NorV-962	FI 962	*Ixodes uriae*		Lofoten, Norway Rost Islands	5—6 July 74	FI 873
Great Island (GI) CanAr 41	GI	*Ixodes uriae* 10 engorged nymphs	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	27 July 71	
Great Island (GI) CanAr 32	GI 32	*Ixodes uriae* 1 engorged female	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	23 July 71	

Table 1 continued (part II)

Great Island (GI) CanAr 40	GI 40	*Ixodes uriae* 10 engorged nymphs	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	27 July 71	
Great Island (GI) CanAr 42	GI 42	*Ixodes uriae* 10 engorged nymphs	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	27 July 71	
Great Island (GI) CanAr 45	GI 45	*Ixodes uriae* 2 unengorged nymphs	Puffin colony (*Fratercula artica*)	Great Island Newfoundland Canada	27 July 71	GI 41
Great Island (GI) CanAr 49	GI 49	*Ixodes uriae* 10 engorged nymphs	Herring gull (*L. argentatus*)	Great Island Newfoundland Canada	27 July 71	GI 41
Great Island (GI) CanAr 176	GI 176	*Ixodes uriae* 8 engorged females	Herring gull (*L. argentatus*)	Great Island Newfoundland Canada	31 July 72	
Kenai (KEN) RML 71-1629	KEN	*Ixodes signatus*	Common murre colony *Uriae aalge*	Gull Island Alaska, U.S.A.	23 June 72	
Mykines (MYK) DenAr 12	MYK	*Ixodes uriae* 5 adult females	Puffin colony (*Fratercula artica*)	Mykines Faerce Islands	5 July 74	
Mykines (MYK) DenAr 7	MYK 7	*Ixodes uriae* 3 adult females	Puffin colony (*Fratercula artica*)	Tindholmur Faerce Islands	22 July 74	
Mykines (MYK) DenAr 8	MYK 8	*Ixodes uriae* 4 adult females	Puffin colony (*Fratercula artica*)	Tindholmur Faerce Islands	22 July 74	MYK 7
Mykines (MYK) DenAr 10	MYK 10	*Ixodes uriae* 5 adult females	Puffin colony (*Fratercula artica*)	Mykines Faeroe Islands	5 July 74	MYK 12
Nugget (NUG) MI-14847	NUG	*Ixodes uriae* 10 nymphs	Penguin colony	Macquarie Island Australia	Jan 72	
Okhotskiy (OKH) LEIV-287KA	OKH 287	*Ixodes putus* = *Ixodes uriae*	Seabird colony	Ariy Kamen Rock Sea Okhotsk U.S.S.R.	—[c]	
Poovoot (POV) RML 57493-71	POV	*Ixodes uriae* pool	Common murre colony *Urial algae*	St. Lawrence Island Alaska	—[d]	

Table 1 continued (part III)

St. Abb's FT363	SA	*Ixodes uriae* 10 engorged nymphs	Seabird colony	St. Abb's Head Scotland	27 July 75	
Tindholmur (TDH) DenAr 2	TDH	*Ixodes uriae* 11 adult females	Puffin colony (*Fratercula artica*)	Tindholmur Faeroe Islands	22 July 74	
Tindholmur (TDH) DenAr 3	TDH 3	*Ixodes uriae* 11 adult females	Puffin colony (*Fratercula artica*)	Tindholmur Faeroe Islands	22 July 74	TDH 2
Yaquina Head 15	YH	*Ixodes uriae* 10 engorged nymphs		Yaguina Head Right Rock Oregon, U.S.A.	Aug 70	
Yaquina Head 59	YH 59	*Ixodes uriae* 10 adult females		Three Arch Rock Oregon, U.S.A.	Aug 70	YH 15
Yaguina Head 62	YH 62	*Ixodes uriae* 10 engorged nymphs		Three Arch Rock Oregon, U.S.A.	Aug 70	YH 15
Yaquina Head 45	YH 45	*Ixodes uriae* 11 engorged nymphs		Yaquina Head Right Rock Oregon, U.S.A.	Aug 70	
Yaquina Head 58	YH 58	*Ixodes uriae* 10 adult females		Three Arch Rock Oregon, U.S.A.	Aug 70	YH 45

[a] Represents the year in which the isolate was collected in the field.
[b] Indicates isolates that had the same PAGE profile as a previously isolated virus.
[c] Virus was isolated from ticks collected from 1969—1971 (Lvov *et al.*, 1973).
[d] Year of isolation not published (Yunker *et al.*, 1973; Yunker, 1975).

RNA probes and blot hybridization. Virus was extracted from infected cells (Ramig *et al.*, 1977). The dsRNA was extracted from the virus, precipitated (Gaillard and Joklik, 1982), and electrophoresed through low melting temperature agarose (Marine Colloids, Rockland, ME) to further purify the dsRNA (Bodkin and Knudson, 1985*b*). The dsRNAs used for probes were 3′ end-labeled with 1.48 MBq of [5′-^{32}P] pCp (Donis-Keller, 1979; Knudson, 1981; Gonzalez and Knudson, 1987*a*) and prepared for hybridization (Bodkin and Knudson, 1986). An aliquot of the probe was electrophoresed in a polyacrylamide gel to determine whether segments were labeled evenly.

Procedures employed in the electrophoretic transfer of RNA and in the hybridization at T_m (RNA)-36 and at T_m (RNA)-20 were described elsewhere (Bodkin and Knudson, 1985*a*, 1985*b*, 1987; Gonzalez and Knudson, 1987*a*).

Results

Gel electrophoresis

The dsRNA polyacrylamide gel (PAGE) profiles of the Great Island serocomplex serotypes were distinct (Figs. 1—3, and data not shown). The overall dsRNA PAGE profile for the isolates was 2-4-3-1. The dsRNA PAGE profiles of multiple isolates of a serotype were not always identical. Isolates with the indistinguishable dsRNA PAGE profiles are identified in Table 1.

RNA-RNA blot hybridization

Isolates from the North and South Pacific did not grow well in BHK-21 cells, and thus, they were not used as probes. Hybridizations were done at T_m (RNA)-36 conditions with one exception. Cape Wrath was hybridized to the serotypes using both T_m (RNA)-36 and T_m (RNA)-20 conditions. Genes 7 and 8 of the Great Island probes did not always label evenly. Thus far, this is the only *Orbivirus* serogroup in which unequal labeling of specific genes has been observed.

The order of migration in PAGE of cognate genes among the Great Island serocomplex viruses was not always the same. For example, reciprocal hybridizations between Cape Wrath and Bauline demonstrated that Cape Wrath gene 3 and Bauline gene 4 were cognates (Fig. 1, and data not shown). Since Cape Wrath and Bauline were variant in two genes and the hybridization signals for Cape Wrath gene 3 and Bauline gene 4 were weaker than the signals for both isolates in variant gene 5, the cognates were identified. Since reciprocal hybridizations were not done between all the isolates, the cognates were not identified between variant genes among the other isolates examined. The variant genes were identified simply by the order of their migration in PAGE (Table 2).

Cape Wrath hybridized strongly to eight genes in the majority of the Great Island serocomplex viruses examined at T_m (RNA)-36 conditions (Fig. 1, Table 2, and data not shown). Cape Wrath hybridized weakly to two genes from the middle group of four genes in the dsRNA PAGE profiles of the isolates. In the blot depicted in Figure 1, the quantity of dsRNA of Kenai, Okhotskiy, and Nugget was less than that of the other isolates; and the lighter signals reflect this quantitative difference rather than sequence heterogeneity between the isolates and Cape Wrath. Cape Wrath hybridized equally to all genes of Okhotskiy and Kenai.

Table 2. Genetic relatedness of Great Island serocomplex viruses[a]

Isolate	Probes						
	BAU	CW	FI 808	GI	GI 32	MYK	TDH
BAU	bau, bau[b]	4,5[c]	4,5	4,5	4,5	4,5	4,5
BAU 63	bau, bau	4,5	4,5	4,5	4,5	4,5	4,5
BAU 128	3,d[d]	3,d	3,d	3,d	3,d	3,d	3,d
BAU 172	bau, bau	4,5	4,5	4,5	4,5	4,5	4,5
CW	3,5	cw, cw	—[e]	3,5	—	—	3,5
FI 808	4,5	4,5	fi808, fi808	4,5	4,5	4,5	4,5
FI 873	bau, bau	3,5	3,5	3,5	3,5	3,5	3,5
GI	d,6	d,6	d,6	gi, gi	d,6	d,6	d,gi
GI 32	d,5	d,5	d,5	d,5	gi32, gi32	d,5	d,5
GI 40	d,5	d,5	d,5	d,5	gi32, gi32	d,5	d,5
GI 42	d,5	d,5	d,5	d,5	gi32, gi32	d,5	d,5
GI 176	3,6	3,6	3,6	3,gi	3,6	3,6	tdl, gi
KEN	d,5	cw, cw	—	d,5	—	—	d,5
MYK	d,d	d,d	d,d	d,d	d,d	myk, myk	d,d
MYK 8	4,6	4,6	4,6	4,6	4,6	myk, myk	4,6
MYK 10	d,5	d,5	d,5	d,5	d,5	myk, myk	d,5
NUG	—	4,6	—	—	—	—	—
OKH 287	3,6	cw, cw	—	3,6	—	—	3,6
POV	—	—	—	3,gi	—	—	3,gi
SA	4,5	4,5	—	4,5	4,5	4,5	4,5
TDH	3,6	3,6	3,6	3,gi	3,6	3,6	tdh, gi
YH 15	—	d,d	—	d,gi	—	—	d,gi

[a] Membranes containing the genome profiles of the isolates were hybridized to 1 μg [5′-^{32}P] pCp-labeled genomic RNA from each of the probes.

[b] Two genes were variant among the middle group of four genes. Variant genes which hybridized strongly to their cognates when they were used as probes and were designated by the name of the isolate which was used as the probe in lower case letters: Bauline (bau), Cape Wrath (cw), FI 808 (fi808), Great Island (gi), GI 32 (gi32), Mykines (myk), Tindholmur (tdh).

[c] Variant genes which hybridized weakly to their cognates in the probe were identified by a number representing the order of their migration in PAGE.

[d] Genes designated d were doublets. Although the signal intensity was reduced the gene which was variant could not be identified.

[e] Isolate was not tested against this probe.

Cape Wrath was hybridized to the blot depicted in Figure 1 at T_m (RNA)-20 conditions (data not shown). Cape Wrath hybridized to all genes of Okhotskiy and Kenai. Cape Wrath did not hybridize to either Bauline or FI 808 gene 4, and it hybridized weakly to both isolates in gene 5. Since variant genes with respect to Cape Wrath in the additional isolates were within a doublet, unique genes for these isolates could not be identified.

Probes of Tindholmur, Great Island, Bauline, FI 808, GI 32, and Mykines hybridized strongly to eight genes in the majority of the Great Island isolates examined (Figs. 2 and 3, Table 2, and data not shown). Reciprocal hybridizations with the Tindholmur and Great Island probes demonstrated that Tindholmur and Great Island hybridized strongly to each other in nine genes

Table 3. Great Island serocomplex in vitro reassortment

Minimum number of segments distinguishable[a]	MOI ratio (PFU/cell) (P1 : P2)	Observed progeny (P1 : P2 : R)	Reassortant genotype (Segment number)[b] 1	2	3	4	5	6	7	8	9	10	N[c]
Great Island (G) X BAU 63 Cross:													
5	11 : 8	9 : 0 : 11	X	G	X	B	X	G	B	B	G	G[d]	
			X	B	G	G	B	X	B	G	B	B[d]	
			X	B	X	B	X	G	B	B	X	G[d]	
			X	G	G	G	X	G	B	G	B	B[d]	
			X	G	G	X	X	G	B	G	X	G	2
			X	B	B	G	X	G	G	G	X	G	
			X	G	B	G	X	G	G	G	X	G	
			X	B	X	B	X	G	B	B	X	B	
			X	B	G	X	X	G	G	G	X	G	
			X	B	X	B	B	X	B	G	X	B	
	23 : 29	14 : 0 : 4	X	G	B	G	X	G	G	G	X	G	
			X	B	G	X	X	G	G	G	X	G	
			X	G	G	X	X	G	G	B	X	X	
			X	G	G	X	X	G	B	G	X	X	
Tindholmur (T) X BAU 63 (B) Cross:													
5	10 : 12	10 : 0 : 7	X	X	X	X	X	T	T	T	T	B	2
			X	X	X	X	X	T	T	T	B	B	2
			X	X	X	X	X	T	B	T	T	T	
			X	X	X	X	B	X	B	B	T	T	
			X	X	X	B	B	X	B	B	B	T	
Tindholmur (T) X FI 808 (F) Cross:													
7	10 : 8	14 : 1 : 2	F	X	F	F	F	F	X	F	X	T	
			F	X	F	F	F	T	X	F	X	T	

[a] The number of segments which have a significantly different mobility when the two parental viruses are electrophoresed in polyacrylamide. Thus, it reflects the number of genes for which the parental origin could be determined in progeny viruses. This represents a minimum number because resolution was often improved using end-labeled dsRNA.

[b] The parental origin of each segment is indicated by a single letter or number abbreviation, and segments of undetermined origin are indicated by the letter X.

[c] N is the number of each genotype observed if greater than one.

[d] Polyacrylamide gel profiles of reassortants indicated are shown in Figure 4.

and that they each hybridized strongly to nine genes of Poovoot and Yaquina Head. Great Island hybridized to nine genes of GI 176 whereas, Tindholmur hybridized strongly to ten genes of GI 176. GI 32 hybridized strongly to all ten genes of GI 40 and GI 42. Bauline hybridized strongly to all ten genes of BAU 63, BAU 172 and FI 873. Mykines hybridized strongly to all genes of MYK 8 and MYK 10.

Gene reassortment

Reassortment occurred between the North American isolates Great Island and BAU 63, between the European isolates Tindholmur and FI 808, and

between BAU 63 and Tindholmur (Table 3). Selected reassortant progeny from the cross between Great Island and BAU 63 are represented in Figure 4. Fewer reassortant progeny were observed from the cross between Tindholmur and FI 808 when compared to the other crosses.

Discussion

The twelve Great Island serotypes examined were distinguishable by their dsRNA PAGE profiles, and multiple isolates of a given serotype were not always identical by their PAGE profiles. Virus isolates with identical PAGE profiles were isolated at the same location during the same collection period.

Hybridization signals at T_m (RNA)-36 indicate greater than or equal to 74 % sequence similarity and singals at T_m (RNA)-20 indicate greater than or equal to 86 % similarity. Although the viruses were isolated from different geographic regions, the isolates are greater than 86 % similar in sequence for the majority of their genes. Cape Wrath exhibited between 74 % and 86 % similarity with gene 4 of Bauline and FI 808 and approached the lower limit of 86 % similarity with gene 5 of both viruses.

The available cross-neutralization data correlate with these hybridization results. For example, GI 32, GI 40 and GI 42 were different from Great Island in two genes by hybridization, and systematic differences have been detected in neutralization tests between these two sets of Great Island isolates (Main *et al.*, 1973). GI 176 was distinct from Great Island in one gene and BAU 128 was distinct from Bauline in two genes by hybridization, and one-way cross-neutralization data has been reported between these virus pairs (Main *et al.*, 1976). In general, the Great Island serocomplex serotypes were distinguishable in one or two genes by hybridization, with one gene demonstrating more variation. The cross-neutralization and hybridization data suggest that one (or both) of the variant genes may encode the neutralization epitope. Recently, gene 5 has been implicated as an encoding sequence for neutralization epitopes (Moss *et al.*, 1987).

Gene reassortment as well as blot hybridization was used to study the relationship of geographic distribution to the sequence and functional relatedness of the Great Island serocomplex viruses. Isolates from different geographic regions and time periods may have greater sequence similarity than multiple isolates from the same geographic area and time period. The 1971 Canadian isolate, Bauline was more related to the FI 873 virus, which was isolated in Norway in 1974, than to the 1971 Canadian isolate, Great Island. Similarly, GI 176 isolated in 1972 was more related to the 1974 Faeroe Island isolate, Tindholmur than to BAU 172. Further, the reassortment data supports the close genetic relationship of the viruses from different geographic areas. Viruses which reassort cognate genes may belong to the same gene pool (Brown *et al.*, 1988*a*, 1988*b*; Gonzalez and Knudson, 1987*b*, 1988). Since the Great Island serocomplex isolates may belong to the same viral gene pool, sequence and functional relatedness of the Great Island viruses could not be correlated with geographic distribution.

Although the different PAGE profiles among the isolates and the variant genes detected may have resulted from genetic drift, the Great Island serocomplex gene pool has remained relatively homogeneous when compared to other *Orbivirus* serogroups. The high level of sequence conservation among the isolates from distinct geographic areas would implicate gene flow as a constraining force against the establishment of local differences in the virus population (Slatkin, 1987). The congregation in large colonies of the avian hosts of *I. uriae* would allow the spread of virus through the local populations, and transoceanic flights between Scotland and Newfoundland of avian hosts would provide the vehicle for dissemination of the virus between different geographic areas (Main *et al.*, 1973; Tuck, 1971). Although it has not been demonstrated conclusively, these data suggest that gene flow is the major force in maintaining the homogeneity of the virus population through the migration of infected birds with their associated ticks enabling sufficient gene flow within the gene pool from different geographic regions.

Although Great Island serocomplex viruses have been isolated from *I. signatus* ticks, these viruses are principally vectored by *I. uriae* ticks. *I. uriae* have been associated with 23 avian species, whereas *I. signatus* have been associated primarily with cormorants. The virus infection rate for *I. signatus* is significantly lower than that of *I. uriae*, and these differences may be related to the susceptibility of the different ticks or avian hosts to the virus (Lvov *et al.*, 1975). *I. uriae*, therefore, is the primary vector responsible for the maintenance of the virus in nature with *I. signatus* possibly being an incidental host.

The extent of genetic diversity within the Great Island serocomplex virus gene pool appears to be similar to other *Orbivirus* gene pools which are vectored by hard-bodied ticks. The CTF isolates which have been examined by blot hybridization (Bodkin and Knudson, 1987; Brown *et al.*, 1988*c*) were associated primarily with one tick vector, and they were relatively homogeneous except for two variant genes. The Kemerovo serocomplex contains two virus types which have diverged in all ten genes and appear to be in the process of speciation (Brown *et al.*, 1988*b*). Members of each type were isolated from the same region, the same tick host, and they have remained relatively homogenous in all ten genes. In contrast, the Great Island serocomplex viruses were isolated from the same tick species, have remained relatively homogeneous in eight genes, but they were isolated in different geographic regions. These data indicate that the tick host may be an additional factor important to the evolution of tick-borne orbiviruses. Since the Kemerovo types appear to be in the process of speciation, the virus types may have evolved extensively as a result of the strong selective pressure of a new tick host rather than as a result of genetic drift due to geographic isolation.

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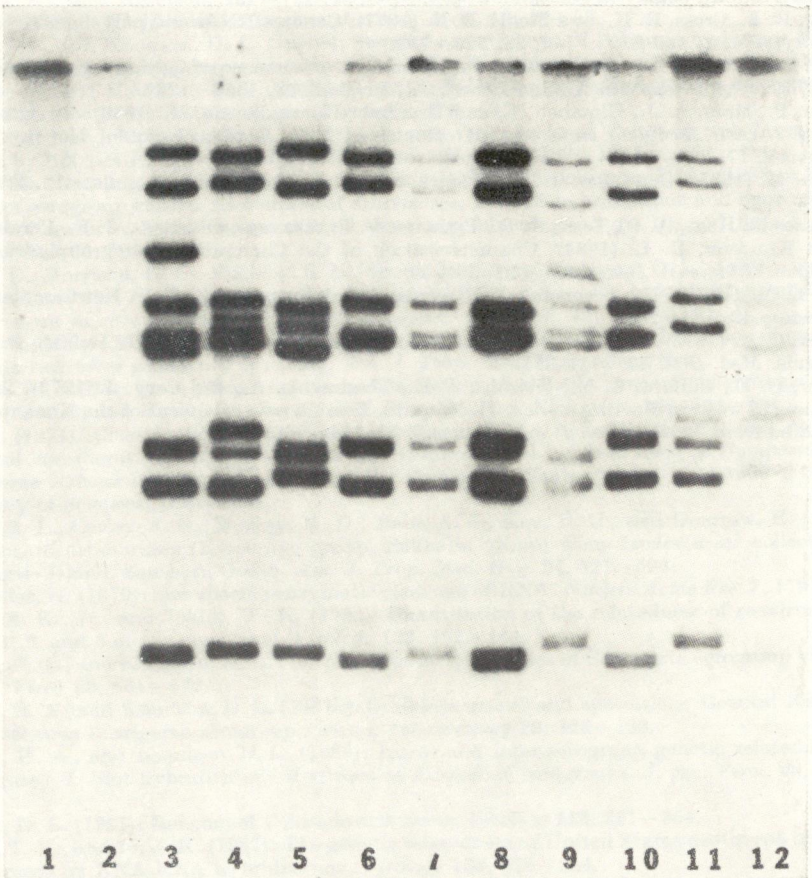

Fig. 1

Autoradiogram depicting the hybridization of the 3′ end-labeled genomic dsRNA probe Cape Wrath to the profiles of representative members of the Great Island serocomplex. RNA was hybridized to the genomic profiles which were electrophoresed through a 10 % polyacrylamide gel at 20 mAmps for 20 hr and transferred to a Zeta-Probe membrane. Lanes are from right to left uninfected BHK-21 cell control (Lane 1), reovirus 3 Dearing strain (Lane 2), Cape Wrath (Lane 3), Bauline (Lane 4), FI 808 (Lane 5), Great Island (Lane 6), Kenai (Lane 7), Mykines (Lane 8), Okhotskiy (Lane 9), Tindholmur (Lane 10), Yaquina Head (Lane 11), and Nugget (Lane 12).

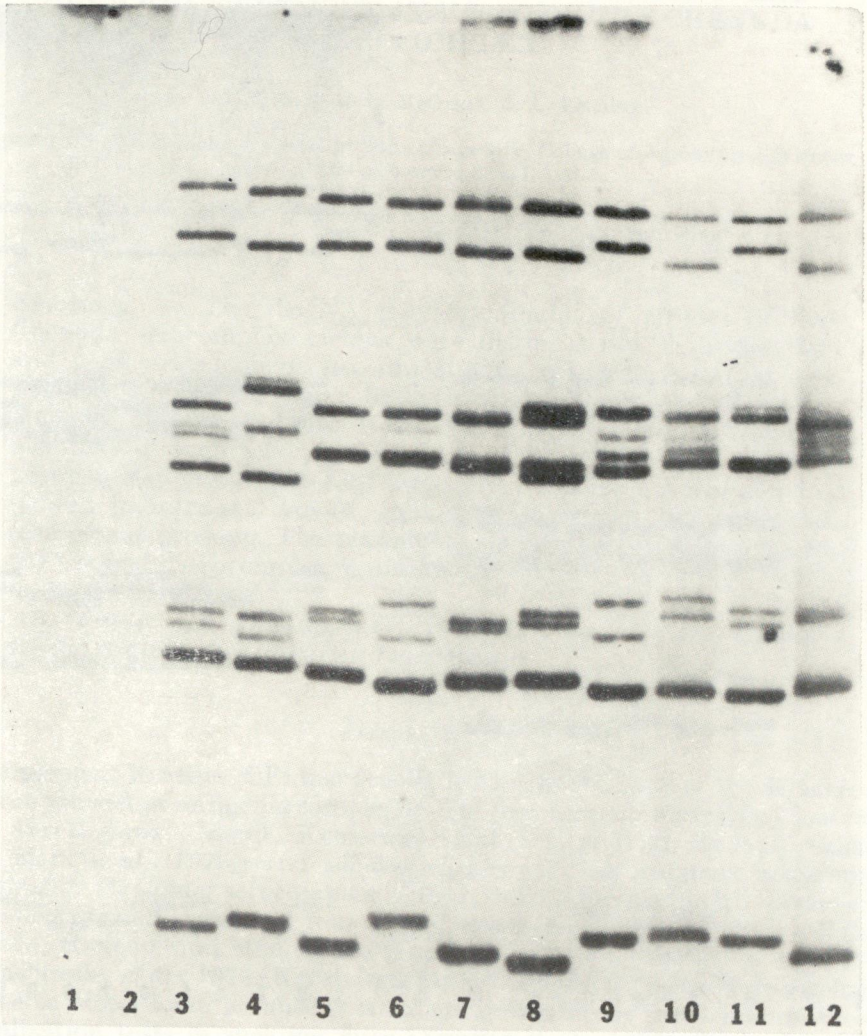

Fig. 2

Autoradiogram depicting the hybridization of the 3′ end-labeled genomic dsRNA probe Tindholmur to a Zeta-Probe membrane containing the profilos of representative Great Island serocomplex viruses. The dsRNA was electrophoresed through a 10 % polyacrylamide gel at 20 mAmps for 30 hr. Lanes are from left to right: uninfected BHK-21 cell control (Lane 1), reovirus 3 Dearing strain (Lane 2), FI 808 (Lane 3), FI 873 (Lane 4), Mykines (Lane 5), MYK 8 (Lane 6), MYK 10 (Lane 7), Tindholmur (Lane 8), Bauline (Lane 9), BAU 63 (Lane 10), BAU 128 (Lane 11), and BAU 172 (Lane 12).

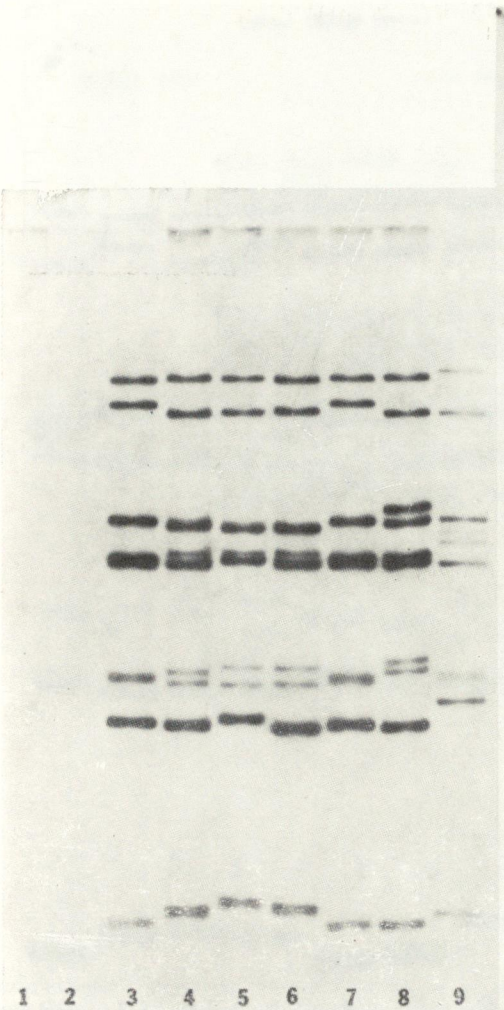

Fig. 3

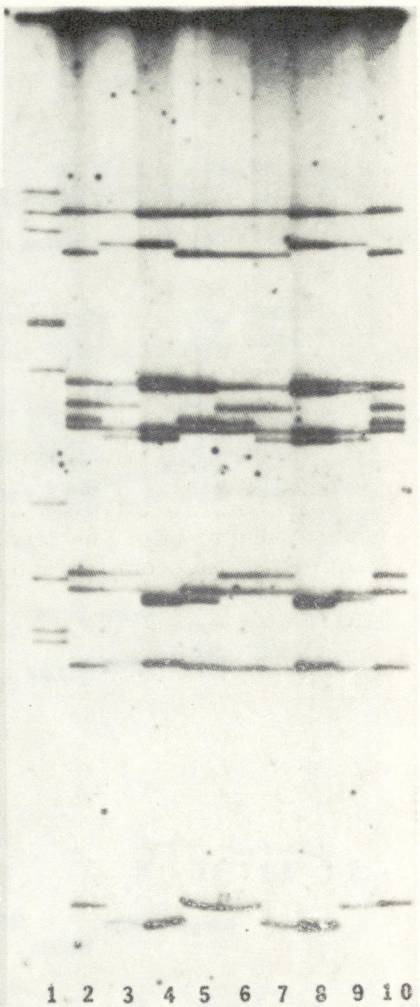

Fig. 4

Fig. 3. Autoradiogram depicting the hybridization of the 3′end-labeled genomic dsRNA probe Tindholmur to a Zeta-Probe membrane containing the profiles of representative Great Island serocomplex viruses. The dsRNA was electrophoresed through a 10 % polyacrylamide gel at 20 mAmps for 30 hr. Lanes are from left to right uninfected BHK-21 cell control (Lane 1), reovirus 3 Dearing strain (Lane 2), Great Island (Lane 3), GI 32 (Lane 4), GI 40 (Lane 5), GI 42 (Lane 6), GI 45 (Lane 7), GI 176 (Lane 8), and St. Abb's (Lane 9).

Fig. 4. Autoradiogram depicting the resolution of the dsRNA genomes of BAU 63, Great Island and selected reassortant progeny. Labeled dsRNA was electrophoresed through a 10 % polyacrylamide gel at 10 mAmps for 20 hr. Each reassortant virus profile is flanked by the two parental profiles for ease in comparison. The genotypes of the reassortants are listed in Table 3. The lanes are from left to right: reovirus 3 Dearing strain (Lane 1), BAU 63 (Lane 2), reassortant BAU 63 X Great Island (Lane 3), Great Island (Lane 4) reassortant BAU 63 X Great Island (Lane 5), BAU 63 (Lane 6), reassortant BAU 63 X Great Island (Lane 7), Great Island (Lane 8), reassortant BAU 63 X Great Island (Lane 9), and BAU 63 (Lane 10).