

A Small Iridescent Virus (Type 29) Isolated from *Tenebrio molitor*: a Comparison of its Proteins and Antigens with Six Other Iridescent Viruses

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SUMMARY

A small iridescent virus (type 29) has been isolated from the meal worm *Tenebrio molitor*. The virus is distinct from a number of previous isolates of small iridescent viruses (types 2, 6, 21, 22, 23 and 28) judged by polyacrylamide gel electrophoresis of its structural polypeptides. Immunodiffusion and immunoprecipitation tests showed that iridescent virus type 29 is related to types 22 and 23 but not types 2, 6, 21 and 28. The relationships between iridescent virus types 22, 23 and 29 were further studied by complement fixation and kinetic neutralization. Complement fixation tests confirmed that these viruses were related and showed that type 29 is distantly related to types 22 and 23 (which were more closely related to each other) though type 23 shares a closer relationship with type 29 than type 22. Kinetic neutralization experiments suggested a close relationship between types 29 and 23, and that both these viruses were remotely related to type 22. Low neutralization rate constants were obtained with these sera.

INTRODUCTION

Recently, an iridescent virus disease of the meal worm *Tenebrio molitor* was found in laboratory stocks of larvae maintained in the Department of Zoology and Entomology, Colorado State University. In the course of virus identification it became obvious that the virus was unusual because it was serologically related to some but not all of a group of small (130 nm) iridescent viruses (types 2, 21, 22, 23, 25 and 28) that had previously been shown to be serologically interrelated. These data, together with electrophoretic analyses of the virus structural polypeptides on SDS-polyacrylamide gels, are reported in this paper.

The virus from *Tenebrio molitor* (designated type 29 according to the scheme of Tinsley & Kelly, 1970) was of interest because it was the first North American isolate of the small iridescent virus group to become available for comparative analysis. Iridescent viruses isolated from invertebrates indigenous to the major continents except the Americas have been shown to be serologically related to some extent, with the exceptions of iridescent virus type 6 (isolated from *Chilo suppressalis*: Fukaya & Nasu, 1966) and possibly iridescent virus type 24 (from *Apis cerana*; Bailey *et al.* 1976), both Asian isolates.

Table 1. *Iridescent viruses used in this study*

Virus	Original host	Country of origin	Authority
IV 2	<i>Sericesthis pruinosa</i>	Australia	Steinhaus & Leutenegger, 1963
6	<i>Chilo suppressalis</i>	Japan	Fukaya & Nasu, 1966
21	<i>Heliothis armigera</i>	Malawi	Carey <i>et al.</i> 1978
22	<i>Simulium</i> sp.	Wales	Batson <i>et al.</i> 1976
23	<i>Heteronychus arator</i>	South Africa	Carey <i>et al.</i> 1978
28	<i>Lethocerus columbiae</i>	Uganda	Carey <i>et al.</i> 1978
29	<i>Tenebrio molitor</i>	U.S.A.	This communication

METHODS

Growth and purification of iridescent viruses. Iridescent virus types 2, 6, 21, 22, 23, 28 and 29 (Table 1) were grown in *Galleria mellonella* larvae and purified as previously described (Kelly & Tinsley, 1972; Elliott *et al.* 1977). Iridescent virus type 29 was isolated as previously described for iridescent virus type 22 (Batson *et al.* 1976).

Serological tests. The methods used in the preparation of antisera, immunodiffusion, immunoprecipitation in free buffer, and complement fixation tests were essentially as described by Elliott *et al.* (1977) and Carey *et al.* (1978).

The immunoneutralization technique consisted of incubating 55 μ l of virus (about 4×10^7 p.f.u./ml: approx. 100 virus particles/p.f.u.) with an equal volume of immune or pre-immune serum (untreated, diluted in PBS where necessary) at 37 °C for pre-determined times (up to 180 min). The samples were diluted 1/100 or 1/300 to stop the reaction and the surviving virus was assayed by plaque assay.

Plaque assay of iridescent viruses was a minor modification of the method described by Brown *et al.* (1978). Neutral red was not incorporated in the primary overlay at the initiation of the assay but added at 5 days as 2 ml of a secondary agarose overlay with 0.03 % (w/v) neutral red. Larger plaques were obtained using this procedure.

SDS-slab polyacrylamide gel electrophoresis followed the method of Laemmli (1970) as previously described (Elliott *et al.* 1977).

Protein estimations were made by the method of Lowry *et al.* (1951) using bovine serum albumen as a standard.

Electron microscopy was performed as described by Gadzama *et al.* (1977).

RESULTS

Pathology of iridescent virus type 29 in Tenebrio molitor

Figure 1 shows a cell infected with iridescent viruses. The particles are about 135 nm in diam. and show morphology typical of small iridescent viruses. Infection foci appeared as cytoplasmic 'factories' encompassing a central 'virogenic stroma' enclosing and surrounded by virus particles. The detailed pathogenesis of this virus will be presented in another report (G. M. Happ, unpublished data).

SDS-polyacrylamide gel electrophoresis of virus structural polypeptides

Figure 2 shows the structural polypeptides of iridescent virus type 29 co-run with six other small iridescent viruses. Each virus has a characteristically different profile. The major structural polypeptide of mol. wt. about 50000 to 55000 varies in apparent size from virus to virus. Curiously, iridescent virus type 23, the virus most closely related to iridescent type 29 virus, possesses a major structural polypeptide which co-electrophoreses with that of

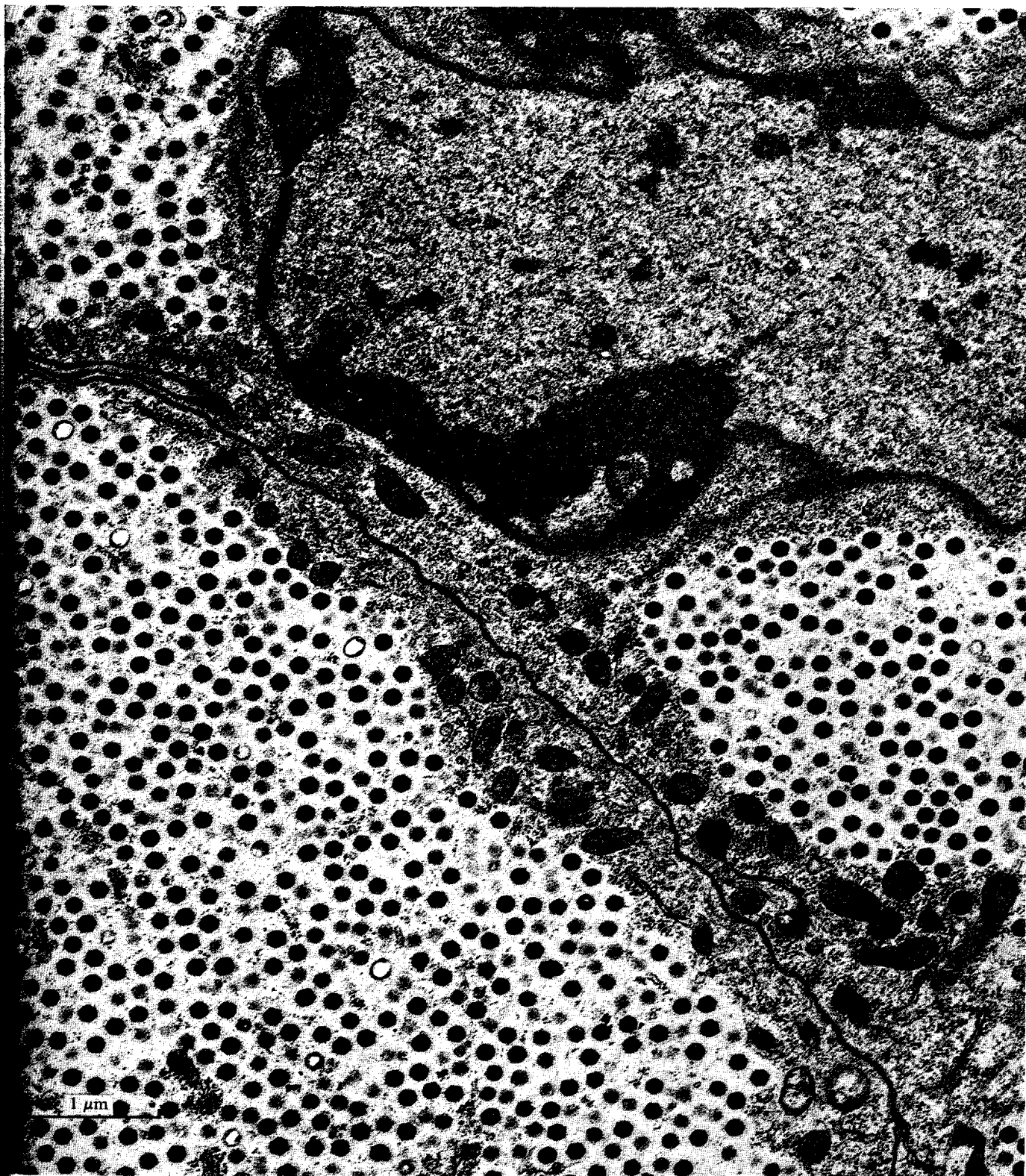


Fig. 1. Electron micrograph of iridescent virus type 29 infected accessory gland in a male *Tenebrio molitor* pupa. Replication occurs in the cytoplasm and there is no effect on the cell nucleus.

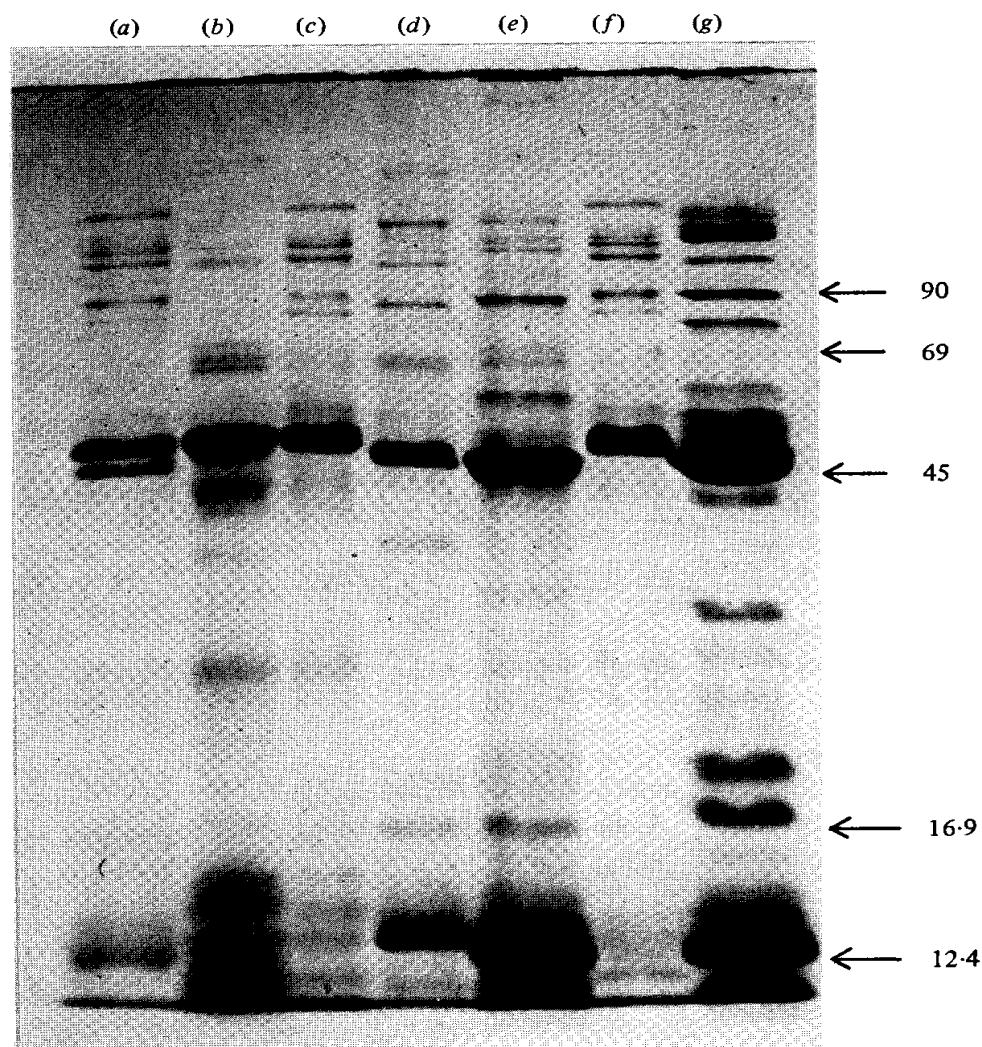


Fig. 2. The polypeptides of iridescent virus types 2, 6, 21, 22, 23, 28 and 29 resolved on a 12% SDS-polyacrylamide gel. (a) Iridescent virus type 2; (b) iridescent virus type 6; (c) iridescent virus type 21; (d) iridescent virus type 22; (e) iridescent virus type 23; (f) iridescent virus type 28; and (g) iridescent virus type 29. Mol. wt. standard migrations ($\times 10^{-3}$) are arrowed.

type 29; the corresponding polypeptide of iridescent virus type 22 (the next most closely related virus) almost co-migrates with the major polypeptides of types 23 and 29, but has a slightly higher apparent mol. wt.; and the remaining four viruses possess a polypeptide of mol. wt. higher than type 22. The extent of phosphorylation and glycosylation of this polypeptide is not yet known and so it would be premature to correlate differences in apparent molecular weight with antigenic differences (since differences in both properties may be affected by these polypeptide modifications).

Comparisons of the viruses by immunodiffusion and immunoprecipitation

The serological relationships of iridescent virus types 2, 6, 21, 22, 23, and 28 with type 29 were examined by comparing the antigens of alkali-disrupted virus in immunodiffusion tests, and the surface antigens of intact viruses in immunoprecipitation tests using antisera prepared against iridescent virus type 29. The results shown in Table 2 demonstrate that only iridescent virus types 22 and 23 share detectable common antigens with iridescent virus

Table 2. Serological relationships between iridescent virus types 2, 6, 21, 22, 23, 28 and 29 assessed by interaction with anti-iridescent virus 29 serum using immunodiffusion and immunoprecipitation

Virus	Serological method	
	Immuno-diffusion*	Immuno-precipitation†
IV 2	0	0
IV 6	0	0
IV 21	0	0
IV 22	1	20
IV 23	1	20
IV 28	0	0
IV 29	3	640

* The number of precipitation lines observed on the plate is shown. One hundred μl of alkali (0.1 M-NaOH) disrupted virus containing 1 mg/ml was allowed to react with a variety of serum dilutions as described by Elliott *et al.* (1977).

† The method of Cunningham & Tinsley (1968) was employed, using 200 μl volumes containing 200 μg of protein and 200 μl of diluted serum. Incubation was at room temperature and the results were scored at 4 h. The values presented are reciprocals of the titres of the serum.

Table 3. Serological relationships between iridescent virus types 22, 23 and 29 assessed by complement fixation tests

Virus	Antisera*		
	IV 22	IV 23	IV 29
IV 22	2048	1024	256
IV 23	512	4096	512
IV 29	128	256	4096

* The microtitre technique of Sever (1962) was employed using 100 μl volumes containing 21.6 $\mu\text{g}/\text{ml}$ of protein and 3HC₅₀ of complement. The values presented are reciprocals of the titre of the serum.

Table 4. Neutralization of infectivity of iridescent virus types 22, 23 and 29 by homologous and heterologous antisera

Antiserum against:	Neutralization constant (K)* measured against:		
	IV 22	IV 23	IV 29
IV 22	0.129 (1.0)†	≤ 0.0005 (≥ 258)	≤ 0.0005 (≥ 258)
IV 23	≤ 0.0005 (≥ 8.6)	0.0043 (1.0)	0.0026 (1.65)
IV 29	0.0016 (8.75)	0.0104 (1.34)	0.0140 (1.0)

* K = fractional rate of neutralization (min^{-1}); 0.0005 was the limit of sensitivity of these experiments.

† Ratio = $K_{\text{homologous virus}}/K_{\text{heterologous virus}}$

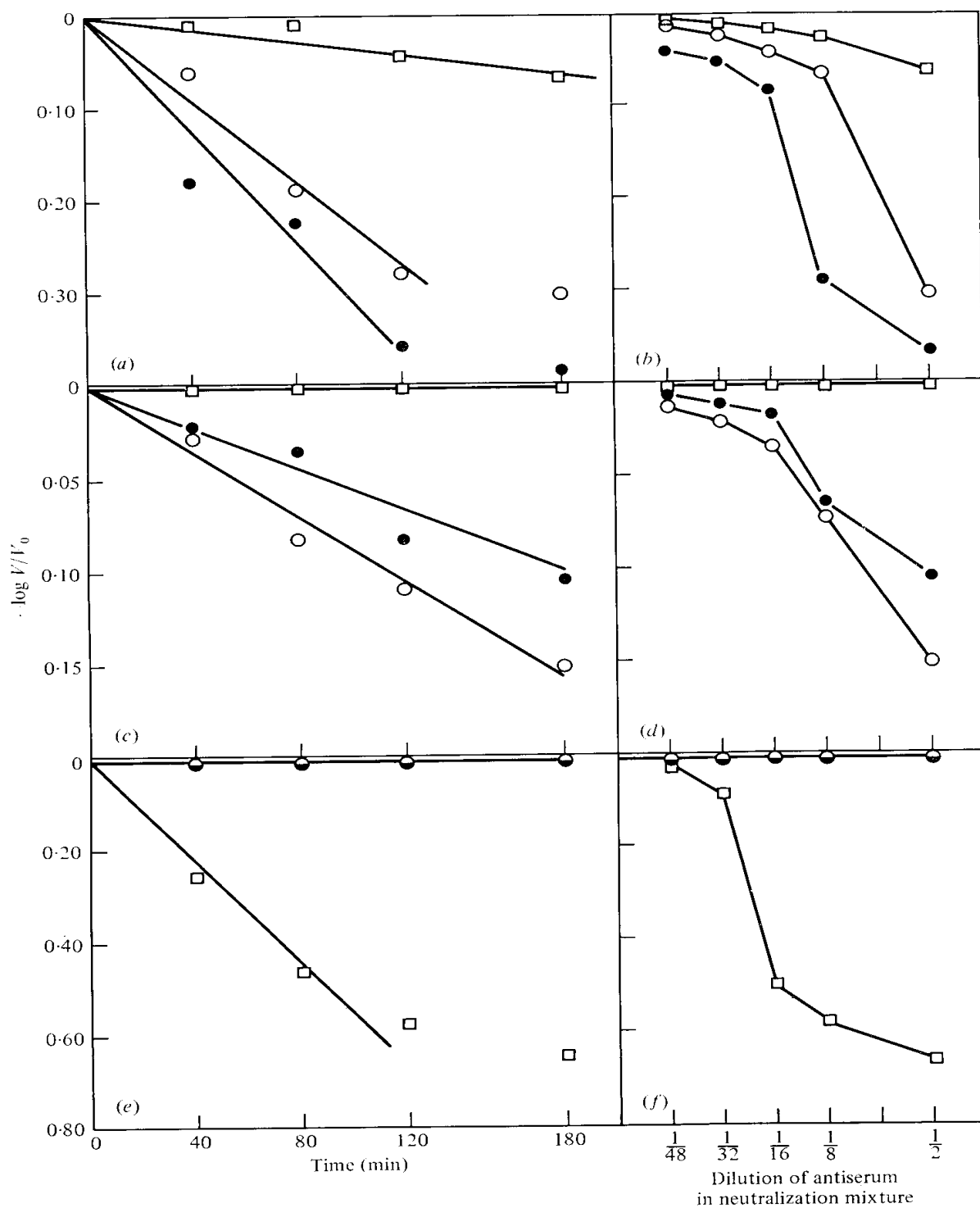


Fig. 3. The kinetic neutralization of iridescent virus types 22, 23 and 29 with respect to time (*a, c, e*) and serum concentration (*b, d, f*). Antiserum to iridescent virus type 29 was used in (*a*) and (*b*); antiserum to iridescent virus type 23 was used in (*c*) and (*d*); and antiserum to iridescent virus type 29 was used in (*e*) and (*f*). ●—●, Iridescent virus type 29; ○—○, iridescent virus type 23; □—□, iridescent virus type 22.

type 29. The two tests indicate that iridescent virus types 22 and 23 are distantly related to type 29 sharing one precipitation line (compared to three in the homologous reaction) and a titre of 1/20 in the heterologous reaction (compared to 1/640 in the homologous reaction) in immunoprecipitation tests.

Comparisons of iridescent virus types 22, 23 and 29 by complement fixation and neutralization

The inter-relationships of these three related viruses were further investigated using the more sensitive methods of complement fixation and kinetic neutralization.

Table 3 shows the serological relationships between these viruses assessed by complement fixation which indicated that iridescent virus type 29 was distantly related to both iridescent virus types 22 and 23, though type 23 shared common antigens with type 29 more extensively than did type 22. Types 22 and 23 were related more closely to each other than to type 29. These results parallel the results obtained by immunoprecipitation confirming observations about these two methods obtained with other iridescent viruses (Elliott *et al.* 1977; Carey *et al.* 1978).

Table 4 and Fig. 3 shows the serological relationships between the three viruses determined by kinetic neutralization, and the results obtained differed somewhat to those obtained by complement fixation. Iridescent virus types 23 and 29 were shown to be closely related to each other but not identical. Both types 23 and 29 were remotely related to iridescent virus type 22. The neutralization rate constants (K) obtained in these experiments were low.

DISCUSSION

The new iridescent virus isolate from *Tenebrio molitor* has unusual serological properties for a small iridescent virus. In its general biological properties – size, morphology, pathogenesis, site of replication, ability to replicate in the wax moth *Galleria mellonella* and overall polypeptide composition – it resembles most other small iridescent viruses (except possibly iridescent virus types 24 and 26 which do not replicate in *Galleria mellonella*). The virus differs from the six viruses used in this study in its detailed polypeptide composition and also differs from these viruses biologically and in its ability to produce considerably larger plaques in *Spodoptera frugiperda* cells than the other viruses (T. Lescott & D. C. Kelly, unpublished observations).

In a previous paper (Carey *et al.* 1978) it was noted that a comparison of the serological inter-relationships between small iridescent viruses is difficult to make. Twenty-four small iridescent viruses have been recorded (Kelly & Robertson, 1973; D. C. Kelly, unpublished data) of which 14 have been compared to varying extents, serologically. The comparative data, based mainly on immunoprecipitation tests made in a number of laboratories, is summarized in Table 5. It can be seen that relationships range from very close to distant, to no relationship. Iridescent virus types 6 and 24 share no common antigens with each other nor any other iridescent virus. Iridescent virus types 1, 2, 9, 10, 16, 18, 21, 22, 23, 24, 25 and 28 all share common antigens to some extent (where tested) and form a broad serogroup. Iridescent virus type 29 is the only small iridescent virus isolate which has been positively shown to share common antigens with some but not all of this broad serogroup. Types 1, 22 and 25; types 21 and 28; and types 9 and 18 cluster as three groups of very closely related viruses. These three clusters of iridescent viruses all contain viruses which can be distinguished when more sensitive serological methods (such as neutralization, complement fixation or enzyme linked immunosorbance assays) are used rather than immunodiffusion or

Table 5. A summary of serological relationships amongst small iridescent viruses*

Iridescent virus type†											Virus and host ^a				
1	2	6	9	10	16	18	21	22	23	24	25	28	29		
+++	+++	-d	++d	++g	++e	nt	+d	+++d	nt	-1	+++d	nt	nt	1 ex <i>Tipula paludosa</i>	
+++	+++	-e	+++	nt	++e	nt	nt	++h	nt	-1	+h	nt	-k	2 ex <i>Sericesithis pruinoso</i>	
+++	+++	+++	-d,e	nt	-e	nt	-d	-d	-d	-1	-d	-d	-k	6 ex <i>Chilo suppressalis</i>	
+++	+++	+++	+++	++g	++e	+++d	nt	nt	nt	-1	nt	nt	nt	9 ex <i>Wiseana cervinata</i>	
+++	+++	+++	+++	+++	nt	nt	nt	nt	nt	nt	nt	nt	nt	10 ex <i>Witlestia sabulosella</i>	
+++	+++	+++	+++	+++	+++	+++	nt	nt	nt	nt	nt	nt	nt	16 ex <i>Costelytra zealandica</i>	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+	nt	+j	+++j	-k	18 ex <i>Opogenia</i> sp.	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	+++h	+++i	+++k	21 ex <i>Heliothis armigera</i>	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	+++j	+++j	+++k	22 ex <i>Simulium</i> sp.	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	nt	+++k	23 ex <i>Heteronychus arator</i>	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	24 ex <i>Apis cerana</i>	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	25 ex <i>Tipula</i> sp.	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-k	28 ex <i>Leithocerus columbiae</i>	
+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	29 ex <i>Tenebrio molitor</i>	

* Data taken from the following sources: ^a Kelly & Robertson (1973); ^b Glitz *et al.* (1968); ^c Kelly (1972); ^d D. C. Kelly & J. S. Robertson (unpublished data); ^e Kalmakoff *et al.* (1972); ^f Kalmakoff & Robertson (1970); ^g Fowler & Robertson (1972); ^h Elliott *et al.* (1977); ⁱ Kelly *et al.* (1978); ^j Carey *et al.* (1978); ^k Kelly *et al.* (this communication); ^l Bailey *et al.* (1976).
 † +++ = serologically very closely related; ++ = closely related; + = related; + + = remotely related; - = no common antigen; nt = not tested.

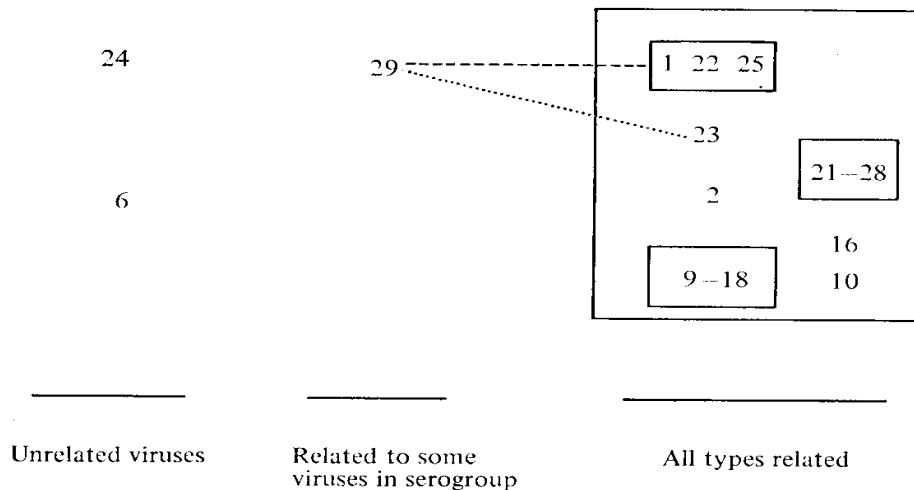


Fig. 4. A schematic representation of the serological relationships between small iridescent viruses.

immunoprecipitation (Elliott *et al.* 1977; Carey *et al.* 1978; Kelly *et al.* 1978). These relationships are illustrated in Fig. 4.

The neutralization rate constants determined for iridescent viruses using rabbit antisera were surprisingly low especially when compared with some animal viruses (Killington *et al.* 1977; Della-Porta & Westaway, 1978) and bacteriophages (Stent, 1963). The sera used in these experiments were of high titre and good avidity when tested by complement fixation or precipitation. Interestingly, two other groups of 'icosahedral cytoplasmic deoxyriboviruses' (ICDVs; Kelly & Robertson, 1973), amphibian ICDVs and African swine fever viruses, also induce the production of high titre complement fixation and precipitin antibodies but low titre neutralizing antibodies in mammalian sera (Came *et al.* 1968; Lehane *et al.* 1968; De Boer *et al.* 1969; Kaminski *et al.* 1969). Consequently, during the course of African swine fever, high levels of viraemia are encountered (Hess, 1971); similarly, the batrachian viruses also elicit high viraemia in amphibia although the nature of the immune response is not known (Clark *et al.* 1969). Iridescent viruses cause lethal disease and do not encounter an immune response in invertebrates and so there is no selective pressure for iridescent viruses to evolve neutralizing antibody resistance as may occur with ICDVs afflicting vertebrates. There is no general explanation why these three groups of ICDVs should all fail to induce a potent neutralizing immune response in mammals. Both the batrachian and invertebrate ICDVs fail to replicate in mammals. In the case of frog virus 3, the best studied batrachian ICDV isolate, the neutralization of the virus is enhanced by supplementing the immune serum with fresh untreated guinea pig serum (Kirn *et al.* 1972) possibly because complement is required in the neutralizing reaction as has been shown with some classes of anti-herpes simplex virus antibody (Yoshino & Taniguchi, 1965). The neutralization of iridescent virus type 22 is being studied in more detail to determine whether the neutralization of iridescent viruses is complement dependent.

No kinetic neutralization experiments have been reported on other groups of invertebrate pathogenic viruses, although the data obtained by Volkman *et al.* (1976) for baculoviruses is more consistent with higher rates of neutralization than those obtained in these experiments.

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