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A New Iridovirus of Two Species of Terrestrial Isopods, *Armadillidium vulgare* and *Porcellio scaber*

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Summary. A new iridovirus, herein named isopod iridescent virus (IIV), was isolated from two species of terrestrial isopods in Berkeley, Calif. Infected individuals of *Armadillidium vulgare* and *Porcellio scaber* (Crustacea: Isopoda) appeared purple in color; in thin sections, hemocytes, nerve cells, and fat body cells contained cytoplasmic aggregations of virions. Isolated particles, 135 nm in diameter in negative stain, contained DNA and included at least 19 polypeptide components. The virus was transmitted per os and by injection to healthy isopods, and by injection to larvae of a coleopteran. IIV from the isopod hosts differed from *Tipula* iridescent virus (TIV) in buoyant density, molecular weights of major polypeptides, and serological reactions with antisera to IIV and TIV.

Iridescent viruses have been found in several phyla of invertebrates, including arthropods, molluscs, annelids, and protozoa [1]. Two recent reports comprise the existing data on iridoviruses in crustaceans: 140-nm particles with typical iridovirus morphologies have been observed in thin sections of a daphnid, *Simocephalus expinosus* [2], and replication of an insect iridovirus (*Chilo* iridescent virus) has been demonstrated after inoculation into two isopod species, *Armadillidium vulgare* and *Porcellio scaber* [3].

In April and May of 1979 natural populations of two species of terrestrial isopods (Crustacea: Isopoda: Oniscoidea) in the hills of Berkeley, Calif., were observed to include purplish-blue individuals as well as the normal ones, which are grey-brown to black. The two species, *A. vulgare* and *P. scaber*, are the most common terrestrial isopods found in this region, *A. vulgare* reaching densities of 500 per square meter [4]. Extracts of the purple isopods examined by electron microscopy

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revealed the presence of many particles with typical iridovirus structure. Preliminary characterization of the virus from each isopod species and comparisons to an iridovirus from *Tipula paludosa* [5] are reported in this paper.

The iridovirus isolates from *A. vulgare* and *P. scaber* were identical in structural appearance, biochemical properties assayed, and serological reactions, and shall be referred to as isopod iridescent virus (IIV).

Materials and Methods

Isopod Rearing and Transmission of IIV

Field-collected isopods of both species have been maintained in the laboratory under ambient conditions of light and temperature for 3 months. Pyrex dishes were filled with a mixture of soil and plant debris with fresh iceberg lettuce as food. In the feeding experiment, 40 laboratory-reared *P. scaber*, each about 5 mm in length, were given lettuce that had been vacuum-infiltrated with 250 μg of purified virus in 2 ml of distilled water as their only food source. 5 *P. scaber* individuals were collected at weekly intervals and assayed for virus content by virus purification followed by measurement of total absorbance at 260 nm. 16 mature *P. scaber*, about 20 mm in length, were chosen for injection; 2 μl of a solution containing 100 μg/ml IIV in distilled water were injected into each animal; 2 isopods were collected at weekly intervals and assayed for virus content by virus purification followed by measurement of total absorbance at 260 nm.

Purification of Viruses

Infected *T. paludosa* larvae, generously donated by Mr. *Fred Wilkinson* of the Canada Agriculture Research Station, Vancouver, B.C., were the source of *Tipula* iridescent virus (TIV), which was purified following the method of *Kalmakoff and Tremaine* [5]. IIV was purified from locally collected purple specimens of *A. vulgare* and *P. scaber*, each species handled separately, using the same procedure. After one cycle of differential centrifugation (2,000 rpm for 10 min in a Sorvall SS-34 rotor followed by 15,000 rpm for 30 min in the same rotor), the virus was further purified by rate zonal density gradient centrifugation in 10–40% sucrose gradients prepared in 0.02 *M* sodium/potassium phosphate buffer, pH 7.3, run for 30 min at 15,000 rpm in a Beckman SW50.1 rotor. Virus concentration was estimated by absorbance at 260 nm in a Beckman model 35 spectrophotometer using the extinction coefficient for TIV ($E^{0.1\%}_{260\,nm} = 18.2$) [5].

Electron Microscopy

Fat body and hemolymph cells were removed from *A. vulgare* and *P. scaber*, fixed, embedded, and processed for electron microscopy [6]. Viral suspensions were incubated on 200-mesh carbon/collodion grids and were then stained with 2% potassium phosphotungstate, pH 6.9. Measurements of IIV diameters were made on photographic enlargements which included latex spheres of known diameter for reference (109 and 312 nm, Ernest F. Fullam, Inc., Schenectady, N.Y.). A Philips EM300 electron microscope was used at 60 kV throughout this study.

Properties of IIV

The sedimentation coefficient of IIV was determined in a Spinco model E analytical ultracentrifuge [7]. Virus (0.5 mg/ml) was centrifuged at 6,166 rpm at 20°, with photographs taken at 4-min intervals using the Schlieren optical system.

Buoyant density determinations in cesium chloride were made by layering 0.2 ml of virus at 1 mg/ml over preformed gradients of 1.25–1.40 g/ml cesium chloride in water, and centrifuging to near equilibrium in a Beckman SW50.1 rotor for 6 h at 45,000 rpm at 10°. The three density species of cowpea mosaic virus were included as standards in a sister gradient. Cesium chloride densities were determined by refractometry; all gradient scanning was done using an ISCO model 640 density gradient fractionator.

Diphenylamine and orcinol tests were performed by standard methods [8].

Analysis of Viral Polypeptides and Nucleic Acid

Viral polypeptides were analyzed in a discontinuous SDS-polyacrylamide slab gel system [9]. Purified virus was dissociated by boiling for 2 min in 1% SDS, 0.1% β-mercaptoethanol, 0.005% bromophenol blue, and 5% glycerol. The polypeptides were electrophoresed for 3 h at 200 V in 10% or 12% slab gels using a Biorad model 220 electrophoresis unit. Molecular weight standards used were phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme (SDS-PAGE molecu-

lar weight kit II; Bio-Rad Laboratories, Richmond, Calif.).

Nucleic acid was isolated from IIV virions by the pronase-SDS method [10]. Cleavage of DNA by *Hin*dIII (Boehringer-Mannheim Biochemicals, Indianapolis, Ind.) and *Pst* I (Bethesda Research Laboratories, Rockville, Md.) restriction endonucleases was performed under the following conditions: 1 μg of IIV DNA was reacted with 1–3 units of enzyme for 4 h at 35° (*Hin*dIII) or 30° (*Pst* I) in the presence of 0.02 *M* Tris, 0.05 *M* NaCl, 0.014 *M* dithiothreitol, and 0.01 *M* $MgCl_2$, pH 7.4 (*Hin*dIII), or 0.02 *M* Tris, 0.01 *M* $MgCl_2$, 0.006 *M* β-mercaptoethanol, and 0.05 *M* $(NH_4)_2SO_4$, pH 7.4 (*Pst* I). DNA fragments were electrophoresed in 0.7% agarose gels in a buffer containing 0.089 *M* Tris, 0.0025 *M* Na_2EDTA, and 0.089 *M* boric acid, pH 8.3, for 3.5 h at 100 V; the gels were stained for 15 min with 1 μg/ml ethidium bromide and were observed under short-wave UV light [11]. Molecular weights were calculated from a standard curve prepared by plotting molecular weight versus mobility of the cleavage fragments of phage lambda DNA treated with *Hin*dIII.

Serology

Antiserum to IIV was prepared in rabbits by injection of purified virus from *A. vulgare*. Rabbits were immunized by an initial intravenous injection followed by two intramuscular injections of virus emulsified with Freund's adjuvant at 2-week intervals. Antiserum to TIV was kindly provided by *J. H. Tremaine* of Agriculture Canada [5].

Antisera were titrated by microprecipitin tests. The alkali degradation procedure [12] was used for immunodiffusion tests. The enzyme-linked immunosorbent assay (ELISA) was used for both detection of IIV and evaluation of serological relationships. The sandwich test [13] was performed with a few modifications [*Morris, Vail and Collier,* manuscript in preparation]; the indirect test [14] proved most effective. Virus was attached to microplate wells by incubation in 0.05 *M* carbonate buffer, pH 9.6, for 3 h at 37°. Plates were washed three times between steps in 0.15 *M* NaCl containing 0.1% Tween. Sera were diluted in phosphate-buffered saline containing 0.05% Tween (PBST) and 2% ovalbumin and were incubated in the wells for 3 h at 37°. Alkaline phosphatase coupled to swine anti-rabbit serum (Bio-Rad Laboratories) was then incubated in the wells at 1–2 μg/ml for 2 h at 37° in PBST containing 2% ovalbumin. The plates were developed by the addition of 1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine, pH 9.8, for 1 h and were recorded at 405 nm.

Results

Pathology of IIV in A. vulgare *and* P. scaber

Infected isopods of both species, made very noticeable by the alteration of coloration, seemed normal in behavior in the field. Approximately 15–20% of the isopods examined were purple in color in May, when population density was highest. Both very young individuals and older, mature isopods (estimated to be 2–3 years old) appeared infected. Normally colored and purple individuals collected in the field could be maintained in the laboratory; after 1–2 weeks all purple isopods died. The healthy, gray isopods have survived for 6 months in the laboratory and have produced offspring. Field-collected gray *A. vulgare* or *P. scaber*, averaging about 75 mg in weight, released no detectable virus by our purification method. Field-collected purple *A. vulgare* weighed about 65 mg and released 0.51 mg of virus per isopod (average of 20); purple *P. scaber* from the field weighed 80 mg and released 0.61 mg of virus per isopod (average of 10).

Hexagonal, iridovirus-like particles were easily located in thin sections of fat body, nerve cells, and hemocytes of embedded purple isopods. Figure 1a shows a degenerating hemocyte of *A. vulgare*. Particles, averaging 115 nm in thin section (average of 27), were found exclusively in the cytoplasm of infected cells, never in nuclei, and accumulated in intercellular spaces. Their appearance was typical of members of the iridovirus group: hexagonal or pentagonal in section, with a capsid enclosing a densely staining core. Many of the virions seemed to have a

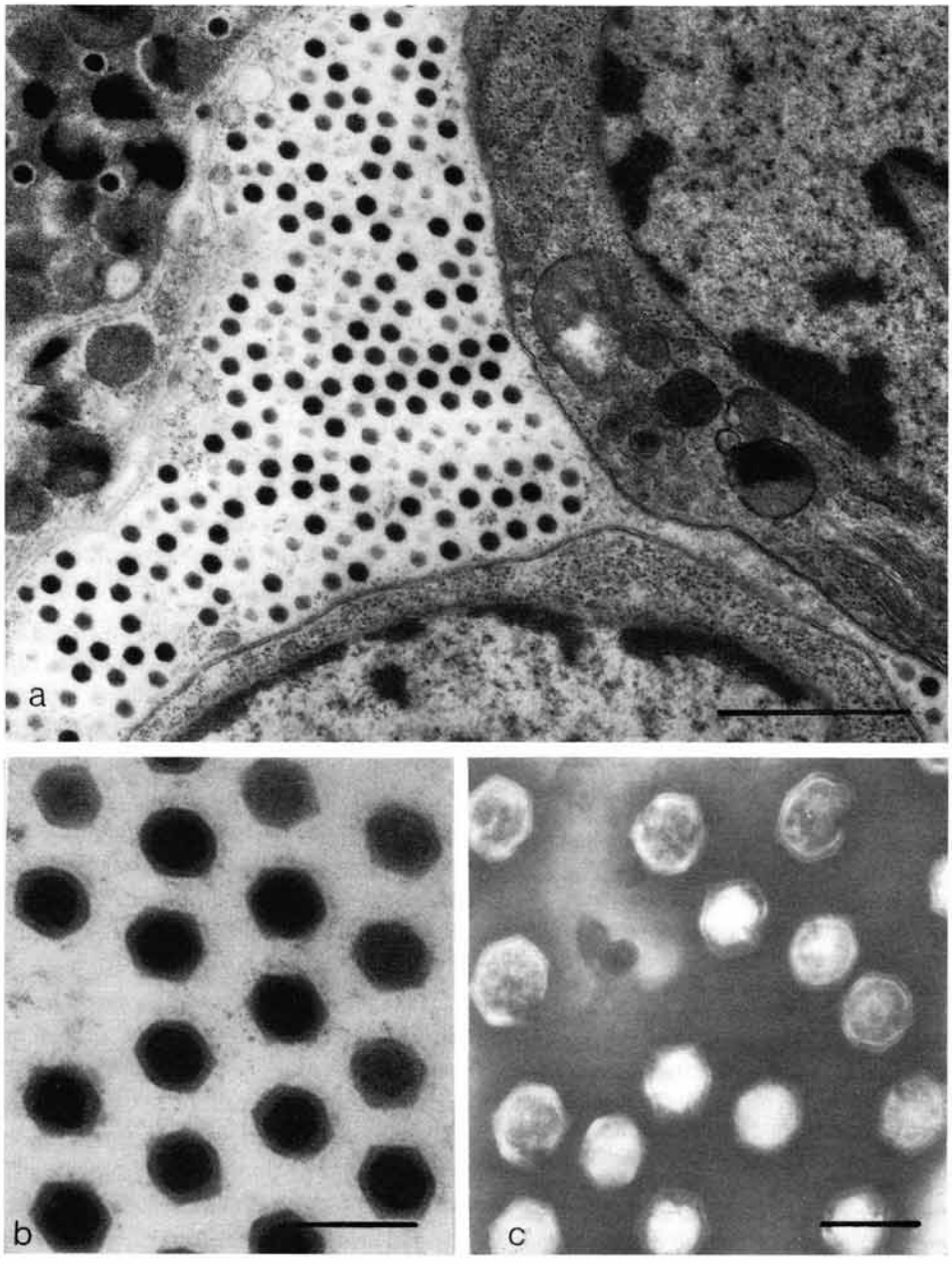

Fig. 1. Electron micrographs of IIV in cells of *A. vulgare* and in negative stain. **a** Hemolymph and fat body cells. Bar = 1μm. **b** Paracrystalline aggregation of intracellular virus, at higher magnification, showing dense cores with hexagonal capsids and a fringe-like configuration around some particles. Bar = 200 nm. **c** IIV particles negatively stained in 2% phosphotungstate, pH 6.9. Bar = 200 nm.

fringe surrounding the capsid (shown at higher magnification in figure 1b), as reported for other members of this group.

Particles liberated by crushing single isopods in buffer, with no further purification steps, are shown in figure 1c. The particles isolated from either species averaged 135 nm in diameter (average of 40) in 2% potassium phosphotungstate stain; particles stained in 2% uranyl acetate were distorted and overstained. No particles were ever seen in squashes of normal-appearing isopods of either species.

Transmission of IIV

Transmission of IIV by feeding or intrahemocoelic injection was readily demonstrated. Young isopods *(P. scaber)*, forced to feed upon IIV-contaminated lettuce, began to develop visible purple coloration in 12 days. Older isopods (2–3 years old *P. scaber*) began to show the symptom 7 days after inoculation with IIV. Virus yields from injected isopods rose to 0.7 mg of virus per isopod. Neither mode of transmission resulted in 100% infection or in synchronous development of symptoms in the isopods tested; however, injection produced more uniform infection. IIV purified from *A. vulgare* readily infected larvae of a soil-inhabiting coleopteran [*Phyllophaga anxia* (Lec.)]. 2 weeks after injection with IIV, the beetle larvae developed purplish coloration; the extracted virus was identical to IIV in polypeptide composition and serological reactions with antisera to IIV and TIV and was infectious to *P. scaber*.

Characterization and Properties of IIV Particles

The method of *Kalmakoff and Tremaine* [5] for purification of TIV proved adequate for IIV. After high-speed centrifugation, pellets

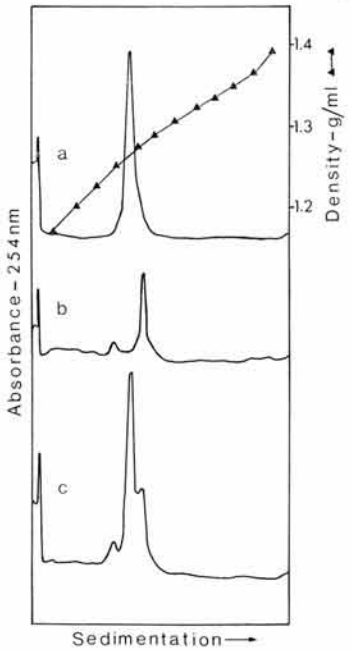

Fig. 2. Sedimentation in CsCl of IIV (a), TIV (b), and a mixture of IIV and TIV (c). Preformed gradients of CsCl in 0.01 *M* Tris, pH 7.5 (1.25–1.40 g/ml) were loaded with virus and centrifuged at 45,000 rpm for 6 h at 10° in an SW50.1 rotor.

of IIV were lavender-blue, while pelleted TIV appeared blue-green. Preparations of IIV were highly light-scattering (67–70% of absorbance at 260 nm) with an $A_{260\,nm}/A_{280\,nm}$ ratio of 1.19:1.21.

A single sedimentable species was consistently isolated from both infected *A. vulgare* and *P. scaber* on sucrose density gradients. A sedimentation coefficient of 2,024 ± 40 S (average of two determinations) was estimated for both isopods, indicating that their sedimentation coefficients were identical.

Sedimentation of virus preparations in preformed cesium chloride gradients further confirmed the identity of the virus isolated from both sources (fig. 2a). Virus was estimated to

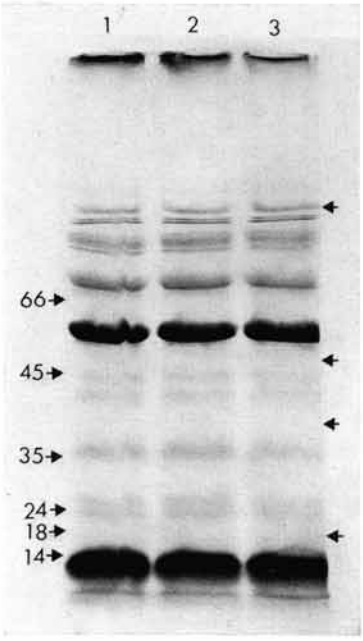

Fig. 3. Polypeptide composition of IIV analyzed on a 12% SDS-polyacrylamide slab gel electrophoresed at 200 V for 3 h at 15°. The gel, from left to right, contains: slot 1, IIV from *A. vulgare*; slot 2, IIV from *P. scaber*; and slot 3, IIV from an *A. vulgare* population in Concord, Calif. The arrows on the left indicate the position of protein standards, and the arrows on the right mark the positions of major TIV proteins.

have a density of 1.262 g/ml in cesium chloride, similar but distinct from the 1.267 g/ml observed for TIV (fig. 2b). A mixture of the two viruses was readily resolved in the gradients (fig. 2c). The density values are somewhat lower than those in the literature because the gradients were not centrifuged to true equilibrium.

Analysis of the polypeptide composition of purified preparations of virus from *A. vulgare* and *P. scaber* revealed essentially identical complex profiles (fig. 3). It was possible to resolve up to 19 protein bands ranging in molecular weights from 11,500 to 126,000 daltons, with a major polypeptide species of 52,000 daltons. The polypeptide profile was clearly different from that of TIV, which had a major species of 46,000 daltons.

The nucleic acid of IIV was DNA. Purified preparations of IIV gave positive reactions in diphenylamine tests and negative reactions in orcinol tests. Purified nucleic acid from IIV virions, visualized by electron microscopy, consisted of long linear strands of the width of double-stranded DNA, but fragmentation of the DNA did not allow accurate estimation of DNA molecular weight. Restriction endonuclease cleavage of IIV DNA by *Hin*dIII and *Pst*I enzymes resulted in the generation of 25 and 15 bands with total molecular weights of 118.7×10^6 and 116.7×10^6 daltons, respectively (fig. 4a, b). DNA from IIV infecting either *A. vulgare* or *P. scaber* gave identical bands after digestion with *Pst*I (fig. 4c).

Serology

The similarity of IIV to other invertebrate iridoviruses prompted a serological comparison to TIV and later to another iridovirus (PIV) isolated from a beetle [*Phyllophaga anxia* (Lec.)] during the course of this investigation. Some evidence for cross-reactivity of IIV to TIV antisera was obtained from droplet microprecipitin tests (TIV antiserum with a homologous reaction of 1:1,024 had a titer of 1:32 against IIV) and alkali immunodiffusion tests. Reciprocal tests using IIV antiserum gave similar results.

The sandwich ELISA test proved most useful for identification of virus-infected individuals but was unable to detect any significant level of cross-reaction between IIV antiserum and TIV. This suggested no more than a distant relationship when compared to the results of *Kelly* et al. [15]. IIV isolated

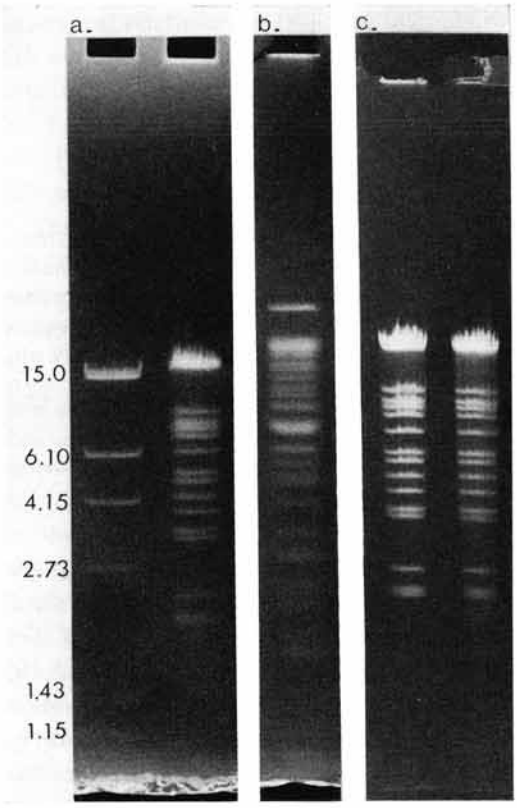

Fig. 4. Restriction endonuclease fragments of IIV DNA stained with ethidium bromide. **a** Slot 1 contains molecular weight markers generated by *Hin*dIII cleavage of 1 μg of phage lambda DNA. Slot 2 represents 1 μg of IIV DNA digested with 1–3 units of *Pst* I endonuclease. **b** Represents 1 μg of IIV DNA digested with *Hin*dIII endonuclease. **c** Slot 1 represents 1 μg of IIV DNA from *A. vulgare*; slot 2 represents 1 μg of IIV DNA from *P. scaber*, each digested with 1–3 units of *Pst* I endonuclease. Mobilities of fragments in views a, b, and c are not directly comparable.

from both *Porcellio scaber* and *A. vulgare* from two locations showed no differences in serological reactions in the sandwich test.

The indirect test [14] proved most valuable for defining serological relationships between the three iridoviruses: IIV, TIV and PIV (fig. 5). Purified virus at several concentrations was adsorbed to microplate wells and then reacted with either IIV antiserum (fig. 5a) or TIV antiserum (fig. 5b) at a dilution of 1:500. Specific binding of antibody was then monitored with enzyme-coupled swine anti-rabbit antiserum. The results clearly indicate a distant serological relationship between TIV and IIV; PIV shows an intermediate relationship with a greater affinity to TIV. This relationship was confirmed by titration of the sera with the three antigens at 1 μg/ml, using the indirect test (table I).

A clear distinction between the viruses could be monitored in a blocking test by incubating bound antigens with test antiserum before reaction with homologous enzyme-coupled γ-globulin. In one such test, at a serum dilution of 1:500, IIV antiserum blocked 81% of the homologous reaction while TIV antiserum blocked only 4.7% of the reactivity.

Discussion

The new virus isolated from two species of terrestrial isopods has properties similar to those of the insect iridescent viruses, including size, architecture, sedimentation coefficient, complex polypeptide composition, DNA content, replication in the cytoplasm, and pathology of the infected host; it clearly falls into this taxonomic grouping.

The same virus strain seems to occur in both isopod species. The virus isolated from either host is identical in size (135 nm in negative stain) and morphology, and accumulation of virus leads to the distinctive purplish coloration. Sedimentation profiles of a mixed preparation of IIV from the two hosts were monodisperse, with only one major peak of

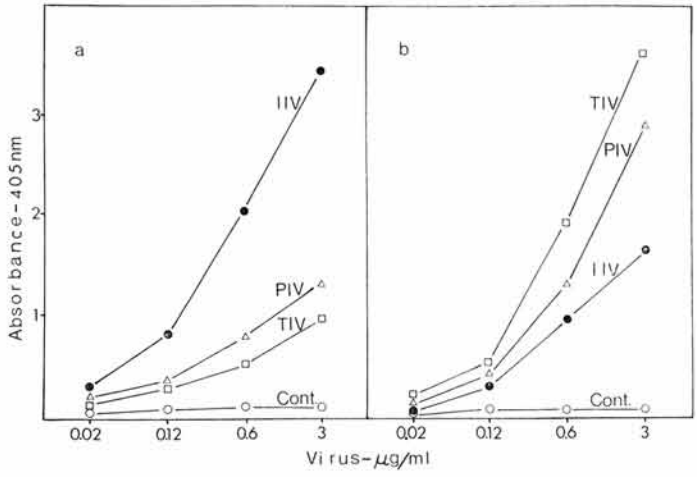

Fig. 5. Serological comparisons of IIV, PIV, and TIV by indirect ELISA. Microplate wells were coated with virus at the concentrations indicated and reacted with IIV antiserum (a) and TIV antiserum (b) at a dilution of 1:500 before the addition of enzyme-coupled swine anti-rabbit antiserum. Cont. = Controls.

Table I. Serological comparisons of IIV, PIV[a] and TIV by indirect ELISA

Antigen[b]	Antiserum titer[c]	
	IIV serum	TIV serum
IIV	10^{-5}	10^{-3}
PIV	10^{-2}	10^{-4}
TIV	10^{-2}	10^{-5}

[a] PIV = *Phyllophaga* iridescent virus.

[b] Microtiter plates coated at 1 µg/ml with purified virus in 0.05 *M* carbonate buffer, pH 9.6.

[c] Serum titer is the highest dilution of serum producing a visible positive reaction (ca. 0.1 absorbance unit at 405 nm).

density in either analytical ultracentrifugation or in cesium chloride density determinations. Electrophoresis of dissociated IIV in SDS-polyacrylamide slab gels resolved 19 polypeptides from virions purified from *A. vulgare* or *P. scaber*. The molecular weight values of the polypeptides were the same, comparing the two host species; in addition, IIV from an *A. vulgare* population located 20 miles east of Berkeley had the same polypeptide composition (fig. 3). The polypeptide components of IIV are clearly different from those of TIV and those of several typed iridoviruses [15, 16]. The patterns of DNA restriction fragments of IIV DNA from *A. vulgare*, produced by cleavage with *Pst*I endonuclease, were identical to those of IIV DNA from *P. scaber* (fig. 4c). IIV from *A. vulgare* replicated in *P. scaber* after an intermediate passage through a coleopteran larval host. No serological differences were detected when comparing IIV isolated from the two isopod hosts.

The serological comparisons between IIV and TIV by several procedures indicated a distant serological relationship. *Kelly* et al., [17] used a sandwich ELISA test to evaluate the relationships between several insect iridoviruses. This method proved ineffective in demonstrating a relationship between IIV and TIV, perhaps because of the distant relationship of the isopod virus. The indirect test, however, allowed for a quantitative study of the relationship, probably because it is not subject to the anomalies of the sandwich test which interfere with evaluation of distant

serological relationships [18]. The inability of TIV serum to block the reaction of IIV and anti-IIV enzyme-coupled globulin would favor the interpretation that the reactivities of the TIV and IIV sera to IIV were for distinctly different antigenic sites.

This iridovirus in terrestrial isopods may constitute an ideal system for study of the ecology and epidemiology of a virus of an invertebrate. The hosts are widespread, occurring in dense populations. Their ecology has been extensively studied [4, 19] and the isopods are easily reared in the laboratory. The iridovirus infection occurs at a high frequency, allowing sampling of relatively small numbers of isopods, unlike the low incidence of infection observed in *Simulium* sp. and *Tipula* sp. [17] and in three African insects [16]. The marker of purple coloration as a measure of frequency of infection probably under-estimates the prevalence of the virus; early stages of infection before enough virus has accumulated to cause iridescence are not detected by the naked eye. The ability to detect virus in crude extracts by ELISA at the nanogram level should facilitate an ecological study on the occurrence of IIV.

The mode of transmission of the iridoviruses from insect to insect is not well understood. Ingestion and wounding have been suggested as routes of virus entry. Ingestion of contaminated diet and injection, mimicking wounding, were both effective modes of transmission of IIV. The isopods are omnivorous scavengers, usually feeding on dead plant material but also on dead invertebrates, including isopods, and mammalian fecal pellets [19]. The possibility that some of these food sources also harbor the virus could be explored using the ELISA technique; of special interest would be neighboring soil invertebrates.

In an attempt to explain the fluctuations of population observed each year of *A. vulgare* living in the Berkeley hills, regulation of population size could not be attributed to food availability or predation, as both these factors stay at a constant level [4, 19]. In a search for a density-dependent regulating mechanism – one which increased in severity with an increase in numbers of individuals – it was concluded that the interaction of weather with other variables, such as shelter site availability, was the important factor. The effect of IIV epidemics on isopod populations should also be taken into account, in view of a 15% visible infection rate at times of peaks of population size. The ELISA technique would be useful in this connection to determine the frequencies of minimally infected isopods at various phases of population density.

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References

1 Fenner, F.: Classification and nomenclature of viruses. Second Report of the International Committee on Taxonomy of Viruses. Intervirology *7:* 1–116 (1976).

2 Federici, B.A.; Hazard, E.I.: Iridovirus and cytoplasmic polyhedrosis virus in the freshwater daphnid *Simocephalus expinosus.* Nature, Lond. *254:* 327–328 (1975).

3 Ohba, M.; Aizawa, K.: Multiplication of Chilo iridescent virus in non-insect arthropods, J. invertebr. Pathol. *33:* 278–283 (1979).

4 Paris, O.H.; Pitelka, F.A.: Population characteristics of the terrestrial isopod *Armadillidium vulgare* in California grassland. Ecology *43:* 229–248 (1962).

5 Kalmakoff, J.; Tremaine, J.H.: Physicochemical properties of *Tipula* iridescent virus. J. Virol. *2:* 738–744 (1968).

6 Hess, R.T.; Summers, M.D.; Falcon, L.A.: A mixed virus infection in midgut cells of *Autographa californica* and *Trichoplusia ni* larvae. J. Ultrastruct. Res. *65:* 253–265 (1978).

7 Markham, R.: A graphical method for the rapid determination of sedimentation coefficients. Biochem. J. *77:* 516–519 (1960).

8 Shatkin, A.J.: Colorimetric reactions for RNA, DNA and protein determinations; in Habel, Salzman, Fundamental techniques in virology, pp. 231–237 (Ccademic Press, New York 1969).

9 Maizel, J.V.: Polyacrylamide gel electrophoresis of viral proteins; in Maramorosch, Koprowski, Methods in virology, vol. 5, pp. 179–226 (Academic Press, New York 1971).

10 Shepherd, R.J.; Bruening, G.E.; Wakeman, R.J.: Double-stranded DNA from cauliflower mosaic virus. Virology *41:* 339–347 (1970).

11 Meyers, J.A.; Sanchez, D.; Elwell, L.P.; Falkow, S.: Simple agarose gel electrophoretic method for the identification and characterization of plasmiJ deoxyribonucleic acid. J. Bact. *127:* 1529–1537 (1976).

12 Cunningham, J.C.; Tinsley, T.W.: A serological comparison of some iridescent non-occluded insect virus. J. gen. Virol. *3:* 1–8 (1968).

13 Clark, M.F.; Adams, A.N.: Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. J. gen. Virol. *34:* 475–483 (1977).

14 Crowther, J.R.; Abu-El Zein, E.M.E.: Detection and quantification of foot and mouth disease virus by enzyme labelled immunosorbent assay techniques. J. gen. Virol. *42:* 597–602 (1979).

15 Kelly, D.C.; Ayres, M.D.; Lescott, T.; Robertson, J.S.; Happ, G.M.: A small iridescent virus (type 29) isolated from *Tenebrio mollitor:* a comparison of its proteins and antigens with six other iridescent viruses. J. gen. Virol. *42:* 95–105 (1979).

16 Carey, G.P.; Lescott, T.; Robertson, J.S.; Spencer, L.K.; Kelly, D.C.: Three African isolates of small iridescent viruses: type 21 from *Heliothis armigera* (Lepidoptera: Noctuidae), type 23 from *Heteronychus arator* (Coleoptera: Scarabaeidae), and type 28 from *Lethocerus columbiae* (Hemiptera Heteroptera: Belostomatidae). Virology *85:* 307–309 (1978).

17 Kelly, D.C.; Edwards, M.L.; Robertson, J.S.: The use of enzyme-linked immunosorbent assay to detect, and discriminate between, small iridescent viruses. Ann. appl. Biol. *90:* 369–374 (1978).

18 Koenig, R.: ELISA in the study of homologous and heterologous reactions of plant viruses. J. gen. Virol. *40:* 309–318 (1978).

19 Paris, O.A.: The ecology of *Armadillidium vulgare* (Isopoda: Oniscoidea) in California grassland: food, enemies, and weather. Ecol. Monogr. *33:* 1–22 (1963).