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Polydnavirus Genome Segment Families in the Ichneumonid Parasitoid *Hyposoter fugitivus*

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Sequences homologous to encapsidated polydnavirus genome segments are routinely detected in parasitoid chromosomal DNA; typically, each viral genome segment hybridizes to a single cognate chromosomal locus. In the present study, we show that in some cases, two or more viral genome segments may hybridize to the same chromosomal locus. Genome segments of this type invariably share a majority of restriction enzyme sites, a fact suggesting derivation from a common template. Families of viral genome segments appear to be relatively common in the *Hyposoter fugitivus* polydnavirus genome.

The polydnaviruses comprise a large group of viruses that share a unique genome structure consisting of multiple segments of double-stranded, circular DNA (5–7, 13). Replicating only in the ovaries of certain parasitic wasps, polydnaviruses are clearly destined for export into parasitized host larvae, in which circular viral genome segments apparently colonize host animals to the benefit of the developing parasitoid eggs and/or larvae (10).

Polydnaviruses are therefore of crucial importance to the wasps that carry them and, in keeping with this observation, we find that these viruses are transmitted vertically through germ line tissue (9, 12), such that all individuals of all affected species carry them; transmission with this degree of efficiency would of course be most readily achieved if the genome were passaged in the form of a provirus. Molecular evidence in support of a chromosomal location for sequences homologous to polydnavirus DNA was first provided by Fleming and Summers (3) for an ichneumonid polydnavirus (ichnovirus) from *Campoletis sonorensis* (CsPV). This work was recently extended to a second ichnovirus (HfPV) from *Hyposoter fugitivus* (17), and to a bracovirus from *Cotesia melanoscela* (11). Evidence derived from genetic experimentation suggests not only that polydnavirus DNA is chromosomally located but also that it is this form of DNA that is responsible for the transmission of polydnavirus genomes (9); these suggestions imply that the circular viral DNAs destined for encapsidation are ultimately derived from the portions of parasitoid chromosomal DNA that are homologous to viral DNA (9, 10). If such is the case, then whatever signals act to trigger DNA replication will presumably act, at least initially, on a linear chromosomal template.

Present evidence strongly suggests that for each polydnavirus genome segment, there typically exists a single cognate locus within the parasitoid genome (2–4, 17). This conclusion is based on hybridization experiments in which male parasitoid genomic DNA is probed with cloned viral DNA segments. When genomic DNA is digested with restriction endonucleases that do not cut a particular viral DNA supercoil, a single off-sized fragment (OSF; i.e., chromosomal DNA not comigrating with viral DNA) is observed; when an enzyme that cuts one (or more) times is used, two OSFs are detected. Such results, which thus far appear typical for polydnaviruses, are regarded as providing presumptive evidence of a single chromosomal locus for whatever probe has been used. Here we describe an unusual variation on this theme: chromosomal sequences homologous to certain viral genome segments were detected, but OSFs specific for each genome segment were not observed; i.e., fewer OSFs than expected were generated by digestion of parasitoid genomic DNA. Our observations suggest that sequences homologous to two or more viral genome segments could in fact reside at a common chromosomal locus.

MATERIALS AND METHODS

Protocols for insect rearing, cloning of genome segments, and other DNA manipulations have for the most part been described elsewhere (6, 8, 12). Hybridization was carried out in 7% sodium dodecyl sulfate (SDS)–0.5 M sodium phosphate buffer (pH 7.2) at 68°C (high stringency) or 42°C (low stringency). Subsequent washes were done in 1% SDS–0.05 M sodium phosphate buffer (pH 7.2). The names of genome segment families are defined by the letter assigned to the smallest member; by convention (7, 17), the letter A would be assigned to the smallest superhelical molecule in the viral genome.

RESULTS

The present study began with experiments aimed at cloning entire viral genome segments from ichnovirus HfPV for use as probes against male *H. fugitivus* DNA (17). In preliminary work, uncharacterized probes derived from shotgun cloning experiments were hybridized to viral DNAs digested to completion with a variety of restriction enzymes. Initially, our major interest lay in the identification and ultimate isolation of viral genome segments containing non-cross-hybridizing sequences, because it was believed that these would be particularly useful in analyzing parasitoid genomic DNA for the presence of virus-specific sequences (17). However, relatively few such genome segments were identified; indeed, approximately two-thirds of all HfPV

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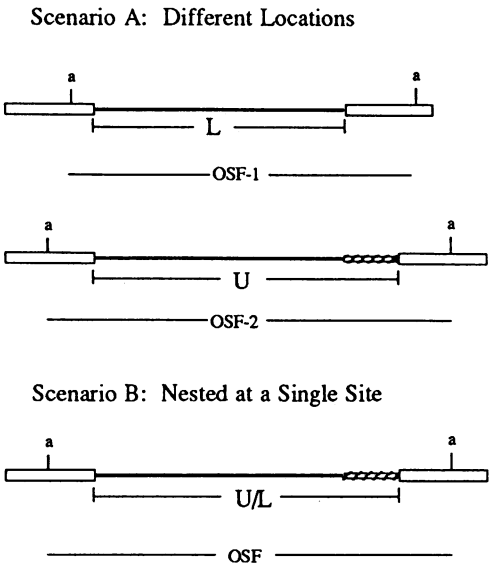

FIG. 1. Schematic representation of OSFs generated by digestion of parasitoid genomic DNA by restriction enzyme a, which cuts neither viral genome segment L nor viral genome segment U (where L and U belong to a family of viral genome segments that are for the most part homologous in terms of shared restriction enzyme sites). In scenario A, sequences homologous to these genome segments are located at different chromosomal sites; in scenario B, the smaller genome segment (L) is nested within U at a common locus. DNA sequences specific for U are indicated by the hatched region; open boxes represent flanking chromosomal DNA.

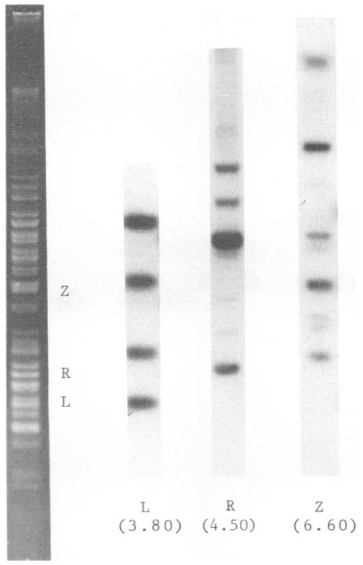

FIG. 2. Genome segment families examined in the present study. On the left are shown viral DNAs following agarose gel electrophoresis and ethidium bromide staining. The positions in the gel of the superhelical forms of genome segments L, R, and Z are indicated. Also depicted are representative lanes from blots of viral DNA probed with the same genome segments, presented in order of increasing molecular mass (kilobase pairs; in parentheses); the blots were probed in each case with the smallest cloned genome segment. Genome segment family L contains two members (both superhelical and relaxed circular forms are seen), R contains three members, and Z contains at least four superhelical forms.

probes examined hybridized strongly with more than one viral genome segment (17). In most cases, however, only two or three genome segments were related (see below); we therefore suspected that the observed cross-hybridization might not have been due to the presence of common tandem repeat elements, such as those described for CsPV (14). M_r data provided no support for the possibility that strongly cross-reacting genome segments were the result of dimerization. Restriction mapping experiments, on the other hand, suggested that most restriction sites identified in related genome segments were in fact shared. In seeking other explanations for an apparent conservation of genetic information, we were led to speculate that our observations could represent presumptive evidence for the existence of families of genome segments, in which closely related members could have been generated by excision from a shared chromosomal template.

Two predictions would logically follow from any such hypothesis. First, if two (or more) viral genome segments have been generated from a common parasitoid chromosomal locus, then only a single OSF should be observed in chromosomal DNA digested by enzymes not cutting the viral genome segments in question (Fig. 1). Second, if the replication of two or more genome segments proceeds from the same chromosomal template, then cross-hybridizing sequences within any such genome segments should be for the most part identical. The results of the present study relate primarily to an examination of the first of these predictions; the second is presently under investigation.

Members of candidate genome segment families identified in the present study are shown in Fig. 2. Families are named with capital letters, such that the family identifier is the letter that has been assigned to the smallest genome segment in the family. The position of the smallest recognized member of each family within the viral genome following ethidium bromide staining is indicated; blots identify all genome segments belonging to each family. We provide a detailed analysis of one family (L) and then present less extensive observations on two others.

Genome segment family L. Genome segments belonging to this family were first discovered when an uncharacterized 4.90-kbp *Hin*dIII clone of HfPV DNA was used as a probe against uncut viral DNA; this probe identified two strongly cross-hybridizing DNAs, at 4.90 and 3.80 kbp. Hybridization at a low stringency did not increase the number of signals observed on autoradiograms (data not shown). Since both genome segments were cut once by *Sal*I, a viral DNA library was constructed by use of this enzyme and *Sal*I clones of the two segments were isolated. The 3.80- and 4.90-kbp *Sal*I clones were named pHfPV-L and pHfPV-U, respectively. When either was used to probe undigested viral DNA, indistinguishable hybridization signals were obtained (data not shown), suggesting that sequences in the smaller genome segment were preserved within the larger; this suggestion was confirmed by Southern blot hybridization and restriction mapping experiments. Observations made with U as a probe are presented in Fig. 3, in which it can be seen that four enzymes (*Eco*RV, *Kpn*I, *Xba*I, and *Xho*I) cut neither genome segment, two (*Hin*dIII and *Pvu*II) cut only the larger circle, two (*Apa*I and *Sal*I) cut both circles once, and several others (*Bgl*II, *Bam*HI, *Eco*RI, and *Pst*I) cut at least one circle more than once. It is evident from Fig. 3 that any enzyme that cuts L also cuts U (e.g., *Apa*I and *Sal*I), while some of those cutting U (e.g., *Hin*dIII and *Pvu*II) do not necessarily cut L; we therefore concluded that all restriction

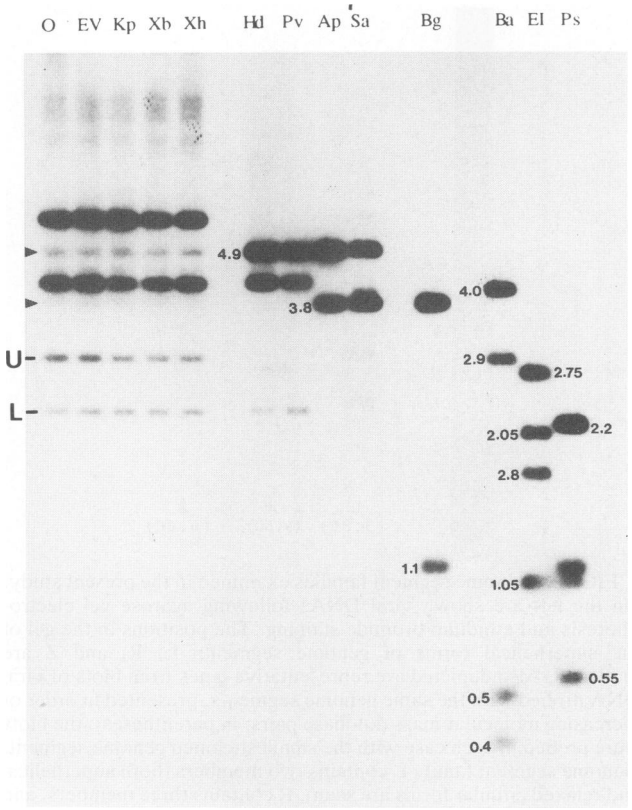

FIG. 3. Genome segment family U. Shown is a Southern blot of viral DNA digests probed with genome segment U. O, no enzyme was used; EV, Kp, Xb, Xh, Hd, Pv, Ap, Sa, Bg, Ba, El, and Ps represent the restriction enzymes *Eco*RI, *Kpn*I, *Xba*I, *Xho*I, *Hin*dIII, *Pvu*II, *Apa*I, *Sal*I, *Bgl*II, *Bam*HI, *Eco*RV, and *Pst*I, respectively. U and L indicate the positions of the superhelical forms of the two genome segments; the arrowheads indicate the positions of linearized L and U molecules (3.80 and 4.90 kbp, respectively). Numbers indicate sizes in kilobase pairs.

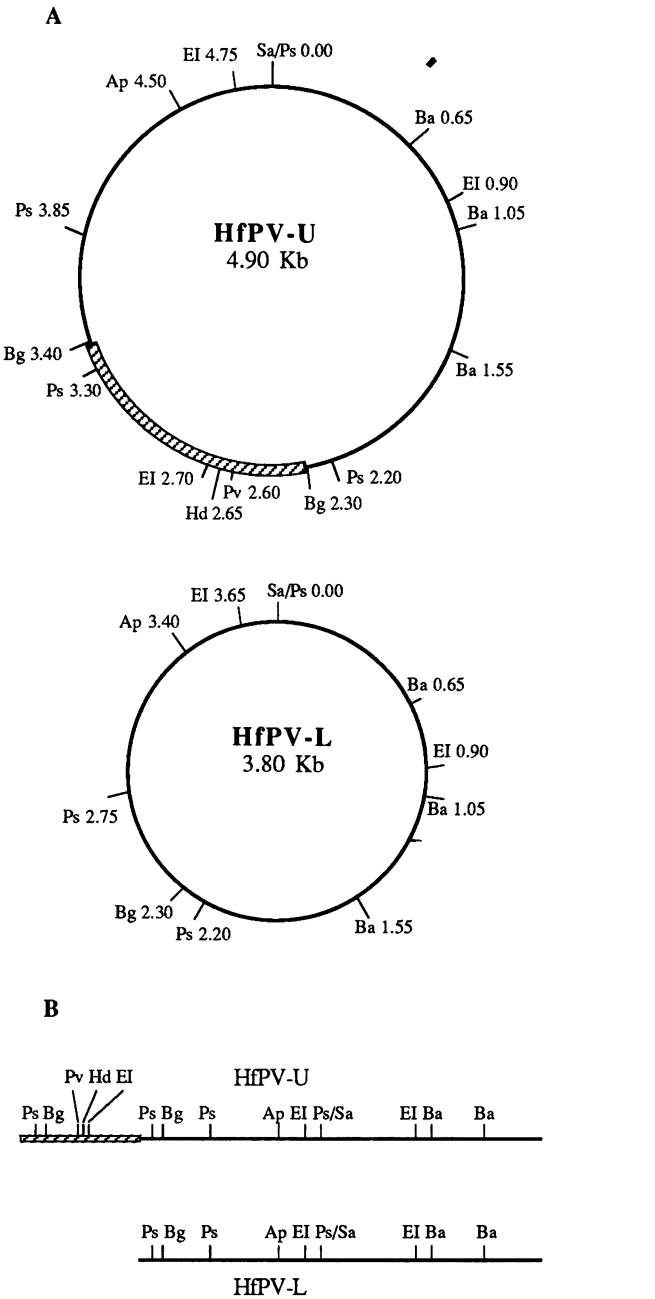

FIG. 4. (A) Circular restriction maps of genome segments U and L. Numbers are in kilobase pairs. (B) Linear alignment of shared restriction sites. The hatched bar represents a 1.10-kbp *Bgl*II fragment containing all restriction sites unique to genome segment U. Restriction enzymes are as defined in the legend to Fig. 3.

sites in L are conserved in the larger genome segment (U), but U contains in addition to these some unique sites. Restriction mapping (Fig. 4) clearly indicates that genome segment L is repeated in its entirety within genome segment U. It should be noted that restriction sites unique to the larger genome segment (U) are entirely contained within a 1.10-kbp *Bgl*II fragment (significantly, this is also true for a *Pst*I fragment of exactly the same size). The larger of the two *Bgl*II fragments is in excess molar ratio, because it contains the homologous 3.80-kbp fragments derived from both genome segments; the smaller *Bgl*II fragment contains all U-specific sites and, at 1.10 kbp, represents precisely the difference in size between L and U. It should be noted that in viral DNA blots probed with the smaller genome segment (L), hybridization signals at both 3.80 and 1.10 kbp were readily detected (data not shown); thus, not all of the 1.10-kbp *Bgl*II fragment is U specific.

By extrapolation from previous work on non-cross-hybridizing genome segments (17), if genome segments L and U are located within the parasitoid genome at different chromosomal sites, then they should be flanked by different chromosomal sequences, so that two OSFs would be generated by digestion with enzymes that do not cut either of the two viral genome segments (scenario A in Fig. 1); similarly, enzymes cutting each genome segment once should generate a total of four OSFs. However, entirely different results were obtained. Four of five noncutting enzymes, for example, generated a single OSF (lanes As, EV, Kp, and Xh in Fig. 5A). *Pvu*II, which cuts only one of the two genome segments (U), generated two OSFs; three would have been expected from genome segments located in separate chromosomal regions (Fig. 1). Similarly, three other enzymes (*Bam*HI, *Bgl*II, and *Eco*RI) generated only two OSFs. These observations, summarized in Table 1, are largely compatible

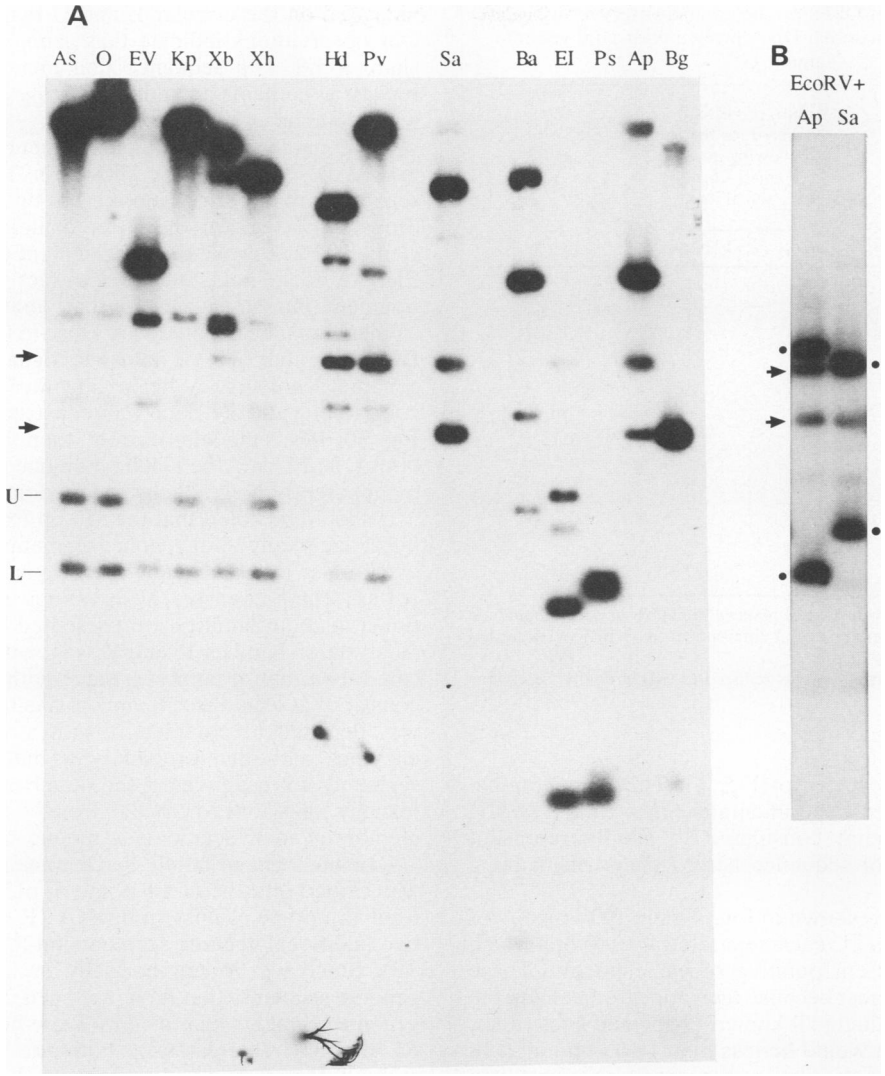

FIG. 5. (A) Southern blot of *H. fugitivus* male genomic DNA digests probed with genome segment U. Note that four of five noncutting enzymes (lanes As, EV, Kp, Xb, and Xh) generate only a single OSF (O, uncut DNA). According to Fig. 1, enzymes cutting each genome segment once (*Apa*I and *Sal*I) should generate two OSFs (scenario B) but here generate more than two. (B) Double digestion with *Eco*RV, however, reduces the number of OSFs (indicated by dots) to two. Further details are provided in the text. L and U indicate the superhelical forms of the two cloned genome segments; the arrows in both panels indicate linearized L and U. Restriction enzymes are as defined in the legend to Fig. 3.

with a scenario (B in Fig. 1) in which the nesting of sequences homologous to the two genome segments occurs at a single chromosomal locus. Results obtained with some enzymes, however, appeared at first to be inconsistent with this interpretation. For example, there is a single site for both *Apa*I and *Sal*I in each of L and U, so that if these genome segments are nested at a single site, two OSFs should be observed on genomic blots; however, three and five OSFs were generated, respectively (Table 1). Similarly, *Hin*dIII cuts only U (once) but nonetheless generates three OSFs instead of the two expected. Earlier work, however, had suggested that some OSFs could result from restriction site polymorphisms in flanking genomic DNA and had revealed that these could often be eliminated in appropriate double-digestion experiments (17). In this regard, it was evident that the faint OSFs seen, for example, in *Sal*I and *Apa*I digests (Fig. 5A) were all relatively large, suggesting that the sites defining these fragments were located well away from L- and U-specific sequences. *Eco*RV, on the other hand, generated a single relatively small OSF, indicating that the *Eco*RV sites in the parasitoid chromosomal DNA were situated close to the L- and U-specific sequences. Accordingly, it was reasoned that double digestions with *Eco*RV in combination with *Apa*I or *Sal*I should eliminate the extra OSFs, reducing the number to the two predicted in each case for a nested scenario; as shown in Fig. 5B, precisely this result was obtained, suggesting that the OSFs observed in *Apa*I and *Sal*I digests were most likely due to the presence of limited restriction site polymorphisms in flanking chromosomal DNA. Taken together, then, the data militate against separate chromosomal sites for sequences cognate to genome segments L and U; indeed, when the double digestions shown in Fig. 5B are taken into account, the results obtained with 10 of 13 enzymes used are fully in

TABLE 1. Numbers of OSFs predicted and observed in digests of *H. fugitivus* chromosomal DNA probed with viral genome segment U[a]

Enzyme	No. of restriction sites in viral genome segment:		No. of OSFs predicted for the following no. of cognate chromosomal loci:		No. of OSFs observed
	L	U	2	1 (Nested)	
*Asp*718	0	0	2	1	1
*Eco*RV	0	0	2	1	1
*Kpn*I	0	0	2	1	1
*Xba*I	0	0	2	1	2
*Xho*I	0	0	2	1	1
*Hin*dIII	0	1	3	2	3
*Pvu*II	0	1	3	2	2
*Apa*I	1	1	4	2	3[b]
*Sal*I	1	1	4	2	5[b]
*Bam*HI	3	3	4	2	2
*Bgl*II	1	2	4	2	2
*Eco*RI	2	3	4	2	2
*Pst*I	3	4	4	2	5

[a] The predictions made were that sequences homologous to viral genome segments L and U were either situated at different chromosomal loci or nested at a single locus (Fig. 1).

[b] The number of OSFs observed was reduced to two in double digestions with *Eco*RV.

accord with a nested scenario (Fig. 1). Those exceptional data not yet addressed experimentally (e.g., three *Hin*dIII OSFs) are at this point considered to simply represent additional examples of sequence heterogeneity in flanking chromosomal DNA.

The restriction maps shown in Fig. 4 identify two pairs of *Pst*I and *Bgl*II sites in U, each separated by 100 bp, which we assumed to represent putative repeat elements. These were of particular interest because they appeared to flank the 1.10-kbp region containing all known U-specific sites (if so, the U-specific domain would be less than 1.10 kbp long). In addition, it appeared that a similar element was present, in a single copy, in the smaller genome segment, L. Since the *Pst*I and *Bgl*II sites in the putative repeat elements were arranged in the same order, it was considered likely that homologous sequences, if present, might well extend beyond those sites. To address these questions, we used three different restriction fragments (A, B, and C; Fig. 6A) as probes for U; the results are shown in Fig. 6B. In one digest (*Pst*I and *Pvu*II; lane 3), probe A recognized two fragments in U, at 0.40 and 0.55 kbp, and probes B and C recognized two additional fragments, at 0.70 and 2.20 kbp. When these hybridization signals were considered in relation to the restriction maps constructed for the two genome segments, it became very clear that U-specific DNA was flanked by repeats of ~500 bp and extending ~250 bp on either side into the 1.10-kbp *Bgl*II (or *Pst*I) fragment which, when added to L, defines U. For example, the observation that probes B and C recognized the 2.20-kbp *Pst*I fragment indicates that sequences to the right of the *Pst*I site at 2.20 kbp on the circular map (Fig. 6) are homologous to sequences to the right of the *Pst*I site at 3.30 kbp (note that these sequences are present in probes B and C but absent from A; Ps 3.30 and Ps 2.20 on maps of U and L, respectively, are equivalent). Careful analysis of these data suggested that sequences unique to genome segment U were restricted to ~600 bp. The repeat elements identified by restriction mapping are indicated on the circular L and U maps shown in Fig. 7A. Our observations indicate that genome segments L and U share a 3.80-kbp sequence that includes one copy of the repeat; U contains an additional copy of the repeat together with ~600 bp of unique sequences.

For construction of a map of sequences cognate to the two nested DNAs, the blot shown in Fig. 5A was probed separately with both L and the 550-bp *Pst*I L fragment (i.e., probe A of Fig. 6); the latter contains largely the repeat element. Each probe recognized all of the OSFs identified in Fig. 5A (data not shown). The fact that the 550-bp *Pst*I fragment did so suggested that one copy of the repeat element must be situated very close to flanking chromosomal DNA on either side of domains cognate to the nested viral genome segments. A linear alignment that appears to be consistent with the data was constructed and is shown in Fig. 7B. If this model is correct, then the 3.80 kbp shared by both L and U and the ~600 bp unique to U are both flanked by apparently homologous repeat domains.

It should be noted that the 550-bp *Pst*I L fragment did not hybridize to any viral genome segments other than L and U, even under low-stringency conditions (data not shown).

Less comprehensive observations relating to two additional genome families are presented below (Fig. 8 to 10). Analysis of families R and Z was somewhat more complicated than that of family L, since both of these appeared to consist of at least three members. As before, genomic DNA was digested to completion with a variety of restriction enzymes and then probed with the appropriate genome segment. In each case, the number of OSFs observed (usually only one or two) strongly suggested nesting of member genome segments at unique chromosomal loci.

Genome segment family R. Genome segment R (4.50 kbp) was cloned into the *Hin*dIII site of pUC19 (Fig. 8A). When used as a probe against viral DNA , R strongly hybridized to two additional genome segments (at 5.50 and 6.60 kbp; Fig. 8B). Since we do not normally assign specific letters to genome segments that have not been cloned, the two larger genome segments identified by R are here simply designated RI and RII. Interestingly, both the superhelical and the relaxed forms of circular RI and RII were consistently underrepresented compared with R. Southern blot hybridization of viral DNA indicated that none of the three genome segments was cut by *Not*I, *Bam*HI, *Apa*I, or *Mlu*I. Analysis of other digests was difficult and consequently is not detailed here; in brief, however, it was found that most restriction enzyme sites were common to all three genome segments, as is evident following linear alignment (Fig. 9A). It should be noted that R, RI, and RII appear to share a common 3.90-kbp *Eco*RI fragment encompassing virtually all of the shared restriction sites; thus, the several hybridization signals observed in uncut viral DNA (Fig. 8B, lane O) were largely reduced to a single signal following digestion with this enzyme (Fig. 8B, lane EI).

When used to probe digests of parasitoid genomic DNA, genome segment R detected relatively few OSFs (Fig. 9B), in keeping with the nested scenario predicted from the linear restriction map alignments. In particular, only one OSF was detected in each case with noncutting enzymes (*Not*I, *Bam*HI, *Mlu*I, and *Apa*I). In most of the other digests, only one or two OSFs were detected. In the *Eco*RI digest, the 3.90-kbp fragment shared by all three genome segments was clearly preserved; this result, together with additional data not shown, suggested that a junction site was probably located somewhere within the 300-bp *Xho*I-*Eco*I fragment (Fig. 8A).

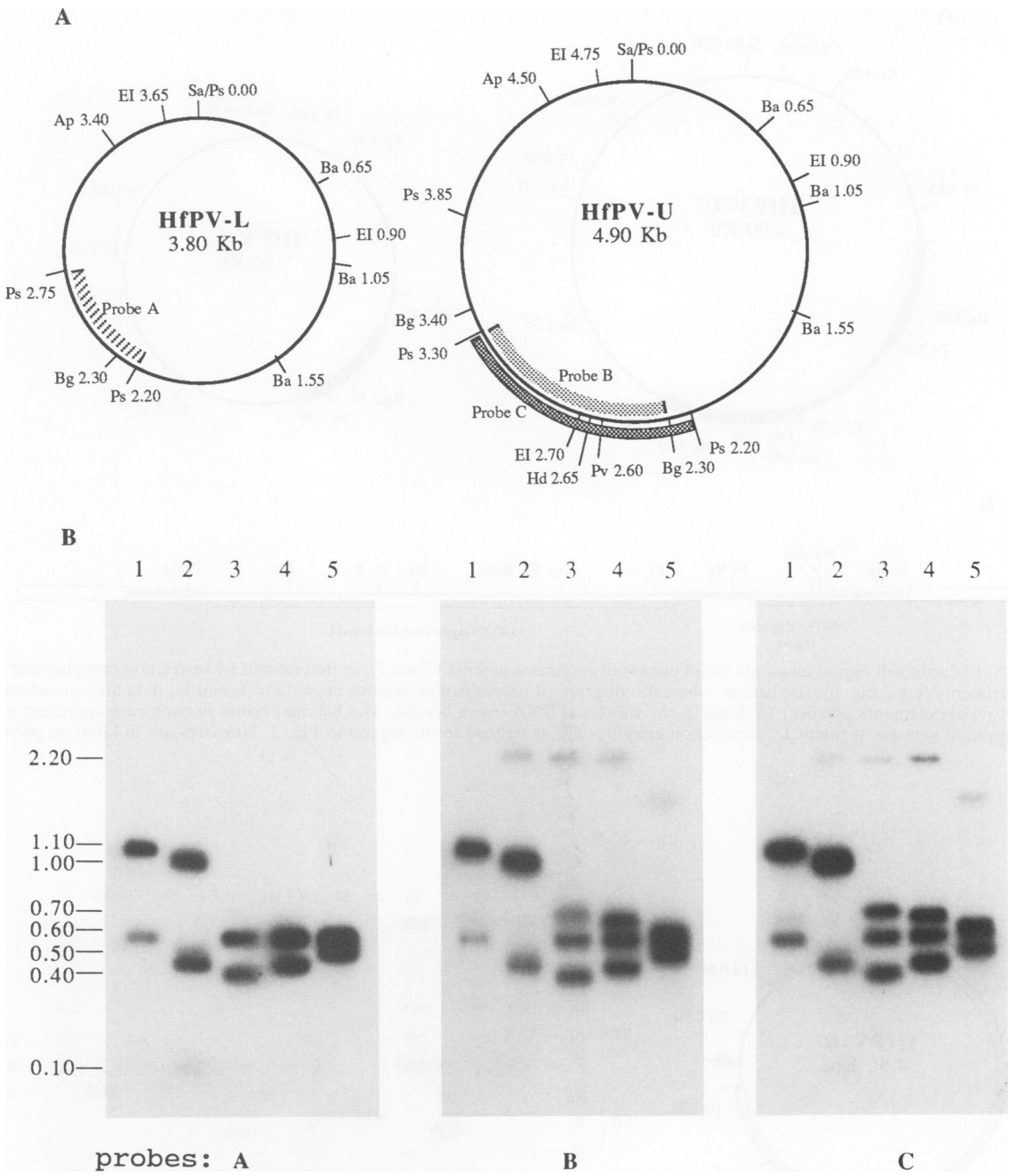

FIG. 6. (A) Locations of probes used to identify repeat elements in genome segments L and U. The 550-bp L fragment (probe A) contains a 100-bp *Pst*I-*Bgl*II element found in two copies in U. Probes B and C encompass a region unique to U. Restriction enzymes are as defined in the legend to Fig. 3. Numbers are in kilobase pairs (Kb). Numbers are in kilobase pairs. (B) Digests of genome segment U probed with the indicated fragments. Lanes 1 to 5 represent double digestions with *Pst*I in combination with *Bam*HI, *Bgl*II, *Pvu*II, *Hin*dIII, and *Eco*RI, respectively. The sizes (in kilobase pairs) of fragments detected by the three probes are provided on the left.

Genome segment family Z. Genome segment Z (6.60 kbp) was cloned at the *Hin*dIII site into pUC19 (Fig. 10A). When used to probe viral DNA, Z hybridized to a set of at least four different genome segments (Fig. 2 and 10B), which remain largely uncharacterized; only two of these routinely hybridized strongly with the probe used. Weak hybridization signals could reflect either a low degree of base sequence homology or an underrepresentation of some of the cross-hybridizing genome segments. In *Eco*RI, *Pst*I and *Pvu*II digests, only two dominant hybridization signals were seen, suggesting that the genome segments involved may share considerable sequence homology; however, hybridization patterns revealed on the blot shown here could not be as readily interpreted as those revealed on comparable blots probed with genome segment U or R. When used to probe chromosomal DNA digested with noncutting enzymes, genome segment Z in each case detected only a single prominent OSF (Fig. 10C).

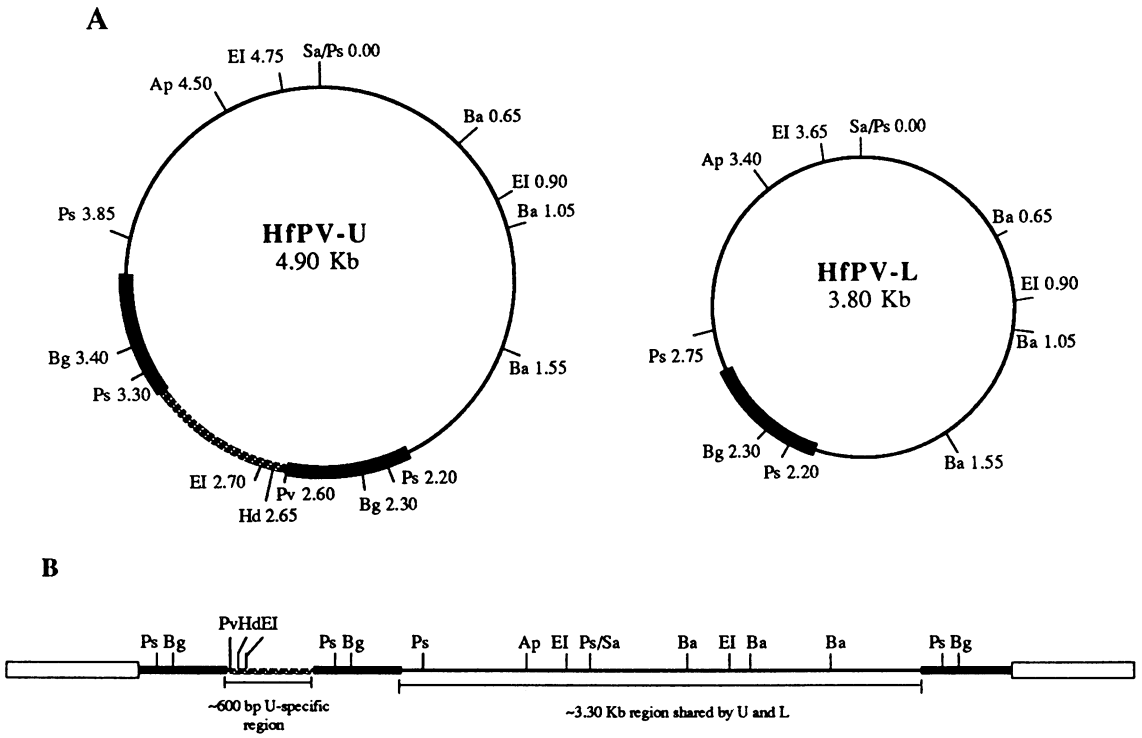

FIG. 7. (A) Locations of repeat elements (solid boxes) in genome segments U and L, as determined by restriction mapping and Southern blotting experiments (e.g., Fig. 6). (B) Linear schematic diagram of chromosomal regions cognate to L and U; data are consistent with the placement of repeat elements adjacent to flanking chromosomal DNA (open boxes). The hatched boxes in each case represent a ~600-bp sequence unique to genome segment U. Restriction enzymes are as defined in the legend to Fig. 3. Numbers are in kilobase pairs (Kb).

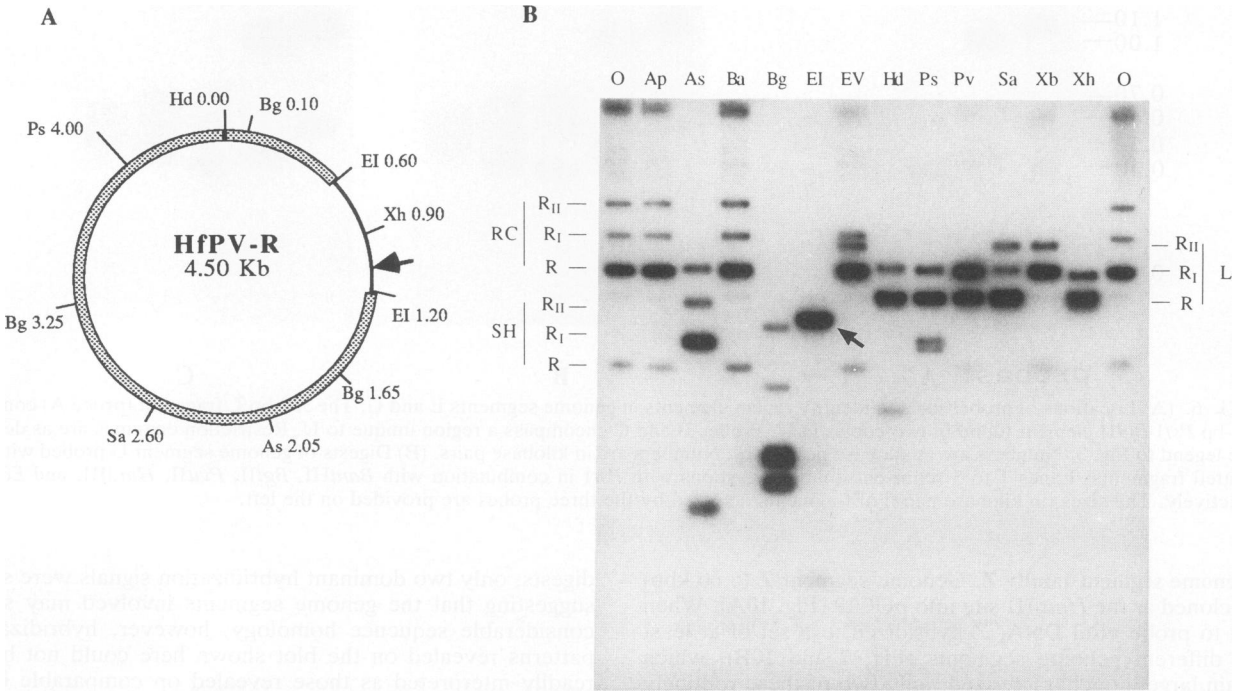

FIG. 8. Genome segment family R. (A) Physical map of genome segment R showing the putative junction site (arrow). Numbers are in kilobase pairs. (B) Southern blot of viral DNA digests probed with R. The positions of superhelical (SH), relaxed circular (RC), and linear (L) forms of the three genome segments are indicated. RI and RII are at 5.50 and 6.60 kbp, respectively. The arrow indicates the position of the 3.90-kbp *Eco*RI fragment mentioned in the text. Restriction enzymes are as defined in the legend to Fig. 3.

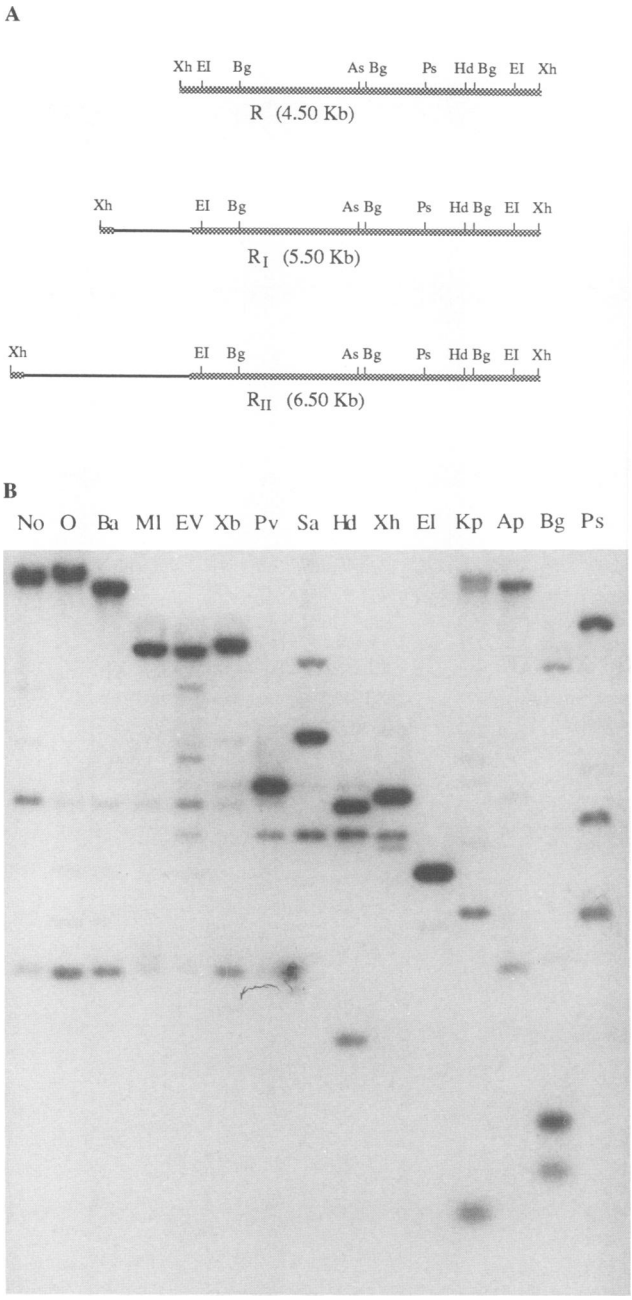

FIG. 9. (A) Linear alignments of R, RI, and RII. Regions containing common restriction sites are hatched. (B) Kb, kilobase pairs. *H. fugitivus* genomic DNA blot probed with R. Restriction enzymes are as defined in the legend to Fig. 3.

DISCUSSION

A polydnavirus genome segment family consists of two or more genome segments that share a majority of restriction sites. As shown here, these common sites can be precisely aligned on linear restriction maps. Restriction digests have further revealed that any site observed in the smallest member of the genome segment family will be found in all larger members of the same family. Given this very characteristic feature, the existence of a genome segment family can often be inferred from a cursory examination of viral DNA blots probed with cloned genome segments, since digestion with some enzymes may be expected to generate a small number (one or two) of hybridization signals (e.g., lane EI of Fig. 8B). These observations indicate that members of a genome segment family may to a considerable extent be homologous in terms of nucleotide sequence, and preliminary sequence data (18) confirm this assumption. This conclusion in turn suggests that members of a genome segment family may be derived from a common template; indeed, in retrospect, it would seem unlikely that assemblages of common restriction sites would have been preserved at two or more separate chromosomal sites. To provide evidence in support of the hypothesis that sequences related to members of a genome segment family may be located at a common chromosomal locus, we probed blots of parasitoid genomic DNA digests with cloned viral genome segments. The resulting hybridization signals were analyzed in terms of the number of OSFs expected in the case of one or more chromosomal sites sharing homology with the viral genome segments used (Fig. 1). In the case of the most carefully examined genome segment family, L, our observations support a model in which sequences homologous to L are nested within a larger U-specific domain at a common chromosomal locus (Fig. 7B and Table 1). Similarly, in two other candidate genome segment families, only a single OSF was typically generated in genomic DNA digests with noncutting enzymes. A polydnavirus genome segment family, then, may be defined as consisting of two or more viral genome segments that are largely homologous both with each other and with a common parasitoid chromosomal locus. Recent work strongly suggests that the structure of the polydnavirus genome is determined by the parasitoid genome; that is, encapsidated circular viral DNAs appear to be generated, at least initially, from linear chromosomal templates (9, 16, 17). While the mechanics of polydnavirus DNA excision and replication remain to be determined, information derived from the present study may provide some useful direction for further work in this area. With the L family as an example, it would seem reasonable to assume that the cognate chromosomal locus would in effect constitute a domain homologous to the largest viral genome segment (U) plus flanking chromosomal sequences. If this scenario is correct, there might exist within this domain a motif that demarcates each of the two nested genome segments (L and U), thus allowing at some point for their separate excision from the shared linear template. Indeed, detailed restriction map analyses strongly suggest the presence of (~500-bp) direct repeat motifs flanking sequences unique to the larger genome segment, U (Fig. 6 and 7); one such motif is also present in genome segment L. Our data also suggest that the putative repeat motifs are situated adjacent to chromosomal domains in the parasitoid genome (since probes encompassing the repeat motifs hybridized to all OSFs). In preliminary work, we have sequenced regions from the two genome segments that include the three repeats (18); approximately half of the regions are identical in all three repeats (the remainder shares more than 95% sequence homology). The presence (and location) of these repeats suggests a model in which L and U could both be generated by homologous recombination from a common chromosomal locus. Terminal repeats adjoining parasitoid chromosomal DNA are also thought to be implicated in the recombinational excision of CsPV genome segment B (4). It should be noted that, in theory, L could also be generated from an extrachromosomal circular form of U by intramolecular recombination; if so, however, a 1.10-kbp circle should also

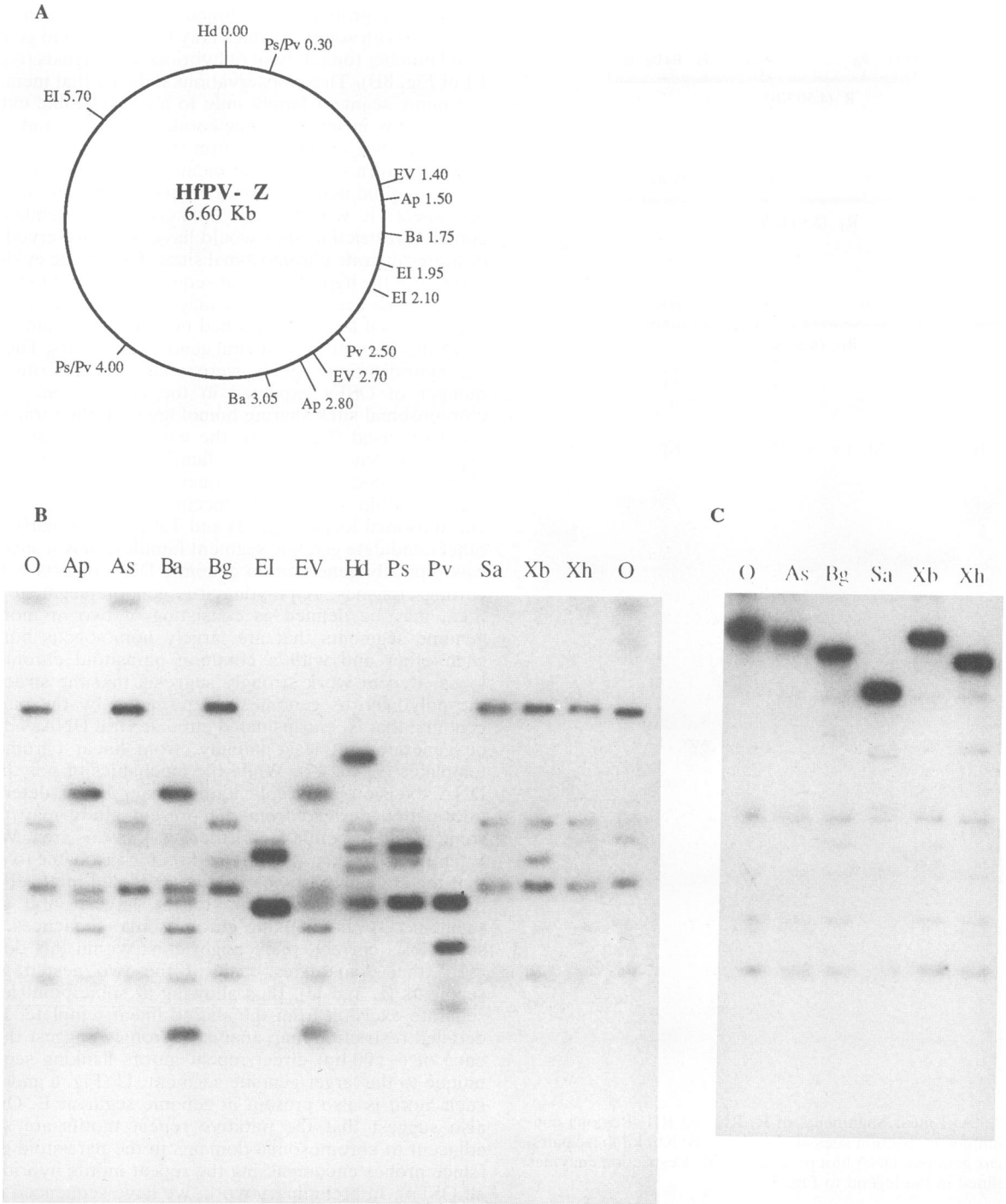

FIG. 10. Genome segment family Z. (A) Circular restriction site map. Numbers are in kilobase pairs. (B) Southern blot of viral DNA digests probed with Z. Note that hybridization signals are reduced to only two dominant fragments following digestion with *Eco*RI, *Pst*I, or *Pvu*II. (C) Blot of parasitoid genomic DNA digested with five different noncutting enzymes; note that only a single dominant OSF is seen in each case. Restriction enzymes are as defined in the legend to Fig. 3.

be generated, but no such genome segment was ever observed, either in an encapsidated form (in purified virus) or in DNA extracted from tissue (wasp ovaries) in which viral genome replication occurs.

It is of interest to examine whether genome segment families represent a feature characteristic of polydnavirus genomes in general; in particular, it is important to consider whether this phenomenon is true for CsPV, which remains to date the only other ichnovirus for which any detailed information on genome structure and organization is available.

Blissard et al. (1) suggested that the CsPV genome might consist of families derived from several master molecules, this having been inferred from observations of cross-hybridization between different genome segments. Theilmann and Summers (14) subsequently reported that cross-hybridization within the CsPV genome was due, at least in part, to the presence of tandem repeats present in most of the genome segments. Genome segments containing these repeats, however, did not share a battery of restriction sites and therefore would not constitute genome segment families as we have defined them. CsPV genome segments containing the tandem repeats described by Theilmann and Summers (14) instead appear to be related by virtue of the fact that they encode a family of related genes (15). At this point, then, it remains to be determined whether genome segment families as such exist in the CsPV genome. We recently obtained preliminary evidence for the existence of at least one such family in a closely related ichnovirus, from *H. lymantriae* (18).

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