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Review Article

Baculovirus: Molecular Insights on Their Diversity and Conservation

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The Baculoviridae is a large group of insect viruses containing circular double-stranded DNA genomes of 80 to 180 kbp. In this study, genome sequences from 57 baculoviruses were analyzed to reevaluate the number and identity of core genes and to understand the distribution of the remaining coding sequences. Thirty one core genes with orthologs in all genomes were identified along with other 895 genes differing in their degrees of representation among reported genomes. Many of these latter genes are common to well-defined lineages, whereas others are unique to one or a few of the viruses. Phylogenetic analyses based on core gene sequences and the gene composition of the genomes supported the current division of the Baculoviridae into 4 genera: *Alphabaculovirus, Betabaculovirus, Gammabaculovirus*, and *Deltabaculovirus*.

1. Background

Baculoviruses are arthropod-specific viruses containing large double-stranded circular DNA genomes of 80,000–180,000 bp. The progeny generation is biphasic, with two different phenotypes during virus infection: budded viruses (BVs), during the initial stage of the multiplication cycle, and occlusion-derived viruses (ODVs), at the final stages of replication [1, 2]. In general, primary infection takes place in the insect midgut cells after ingestion of occlusion bodies (OBs). Following this stage, systemic infection is caused by the initial BV progeny [3, 4]. And finally, OBs are produced during the last stage of the infection. These OBs comprise virions embedded in a protein matrix which protects them from the environment [5, 6].

Baculoviruses have been used extensively in many biological applications such as protein expression systems, models of genetic regulatory networks and genome evolution, putative nonhuman viral vectors for gene delivery, and biological control agents against insect pests [7–17].

The Baculoviridae family is divided into four genera according to common biological and structural characteristics: *Alphabaculovirus*, which includes lepidopteran-specific baculoviruses and is subdivided into Group I or Group II based on the type of fusogenic protein, *Betabaculovirus*, comprising lepidopteran-specific granuloviruses, *Gammabaculovirus*, which includes hymenopteran-specific baculoviruses, and finally *Deltabaculovirus* which, to date, comprises only CuniNPV and possibly the still undescribed dipteran-specific baculoviruses [1, 18–20].

The comparison between known genome sequences of all baculoviruses has been the source for identifying a common set of genes, the baculovirus core genes. However, there are probably more orthologous sequences that may not be identified due to the accumulation of many mutations throughout evolution. Thus, core genes seem to be a key factor for some of the main biological functions, such as those necessary to transcribe viral late genes, produce virion structure, infect gut cells abrogate host metabolism and establish infections [21–24].

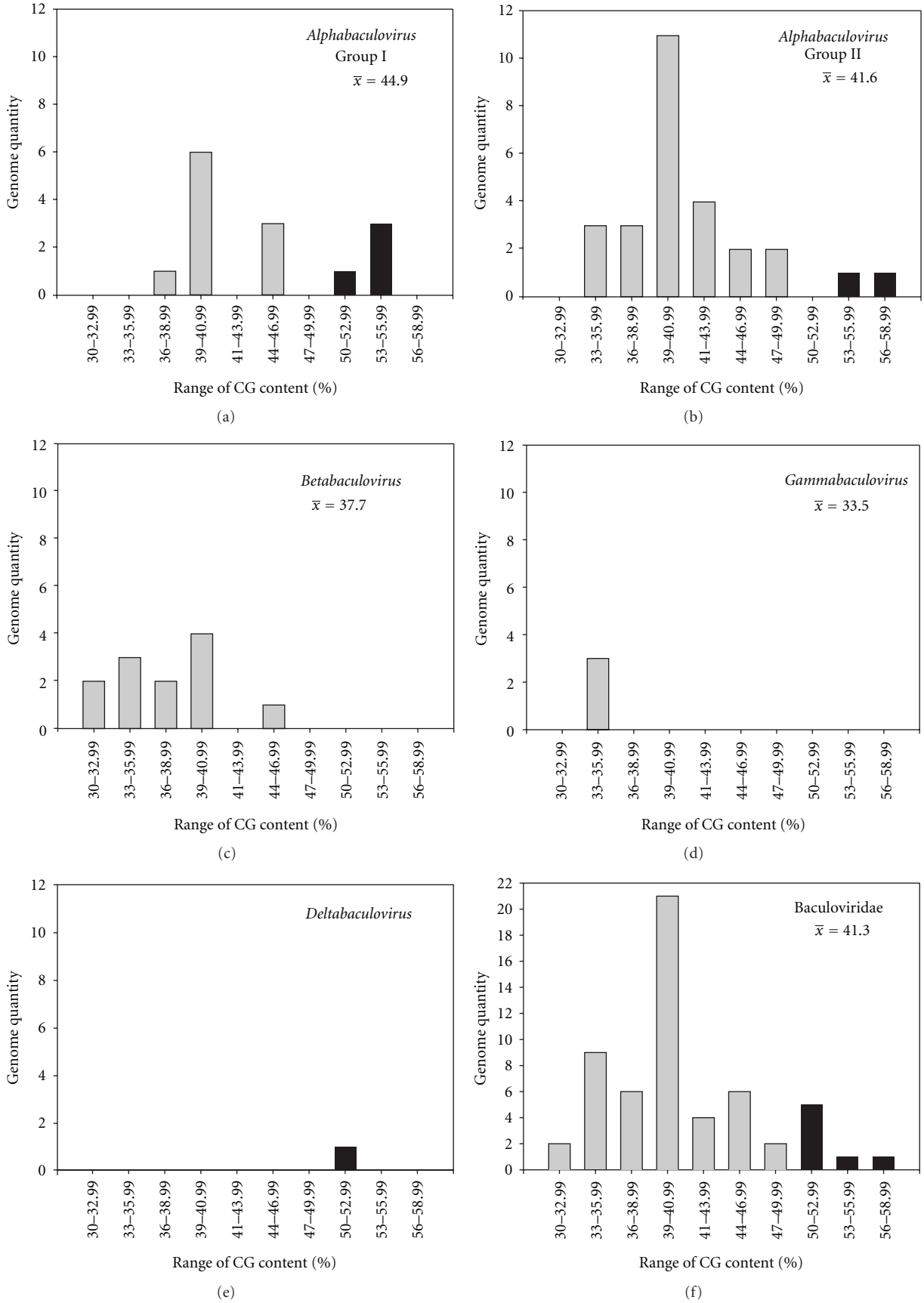

Figure 1: GC content in baculovirus genomes. The different histograms contain the distribution of baculovirus genomes according to their GC content and their genus classification. Black bars highlight genomes with a GC content higher than 50%.

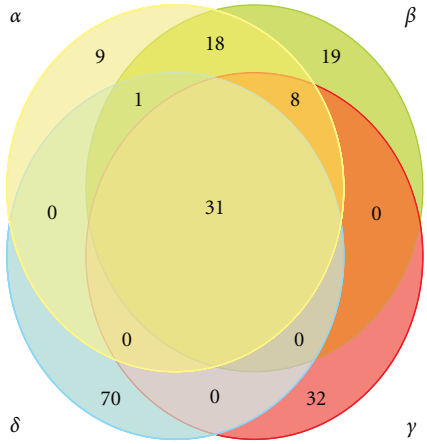

Figure 2: Baculovirus core genes. The different circles represent the 4 baculovirus genera (in yellow *Alphabaculovirus*; in green *Betabaculovirus*; in red *Gammabaculovirus*; in blue *Deltabaculovirus*). The numbers contained within the overlapping regions indicate the amount of shared genes between all members of the genera. The numbers within the circles but outside the overlapping regions indicate the amount of genes shared by all members of that genus but with the absence of orthologous sequences in the remaining genera. These estimations were inferred by Blast P algorithm (http://www.ncbi.nlm.nih.gov/) considering $E = 0.001$ as cutoff value and comparing all reported baculovirus ORFs between them. The identity of common genes is provided in the Supplementary data available at doi:10.4061/2011/379424

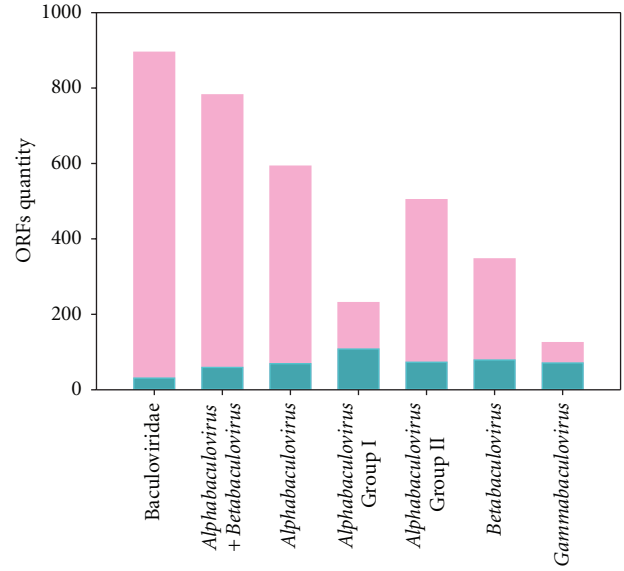

Figure 3: Whole baculovirus gene content. The histogram shows the amount of different reported genes in each baculovirus genus or recognized lineage (bars in pink color), and the subset of shared genes for all members of the corresponding phylogenetic clade (bars in green color). This bar graph was performed using the information resulting from the comparison of all ORFs reported in the 57 baculovirus with known genomes, analyzing all against all by Blast P algorithm (http://www.ncbi.nlm.nih.gov/) considering $E = 0.001$ as cutoff value.

For this report, previous data as well as bioinformatic studies conducted on currently available sets of completely sequenced baculovirus genomes were taken into account and have resulted in a summary of gene content and phylogenetic analyses which validates the classification of this important viral family.

2. Baculovirus Ancestral Genes

There are currently 57 complete baculovirus genomes deposited in GenBank (Table 1). These include 41 *Alphabaculoviruses*, 12 *Betabaculoviruses*, 3 *Gammabaculoviruses*, and 1 *Deltabaculovirus*.

As a first approach to perform a comparative analysis, the GC content of the genomes were calculated (Figure 1). The histogram revealed that many baculoviruses have about 41% of GC content although several of them have significantly higher values (CfMNPV at 50.1%, CuniNPV at 50.9%, AnpeNPV-L2 at 53.5%, AnpeNPV-Z at 53.5%, LyxyNPV at 53.5%, OpMNPV at 55.1%, and LdMNPV at 57.5%). A detailed analysis of DNA content did not show a clear pattern of GC content that could be associated with each genus.

Further characterization of the patterns of gene content and organization may prove useful for establishing evolutionary relationships among members of Baculoviridae. The high variability observed in the number of coding sequences becomes a key feature of viruses with large DNA genomes that infect eukaryotic cells [18]. Insertions, deletions, duplication events, and/or sequence reorganizations by recombination or transposition processes seem to be the main forces of the macroevolution in this particular kind of biological entities. For example, the loss or gain of genetic material could provide new important abilities for colonization of new hosts, or they could improve performance within established hosts. However, there seems to be a set of core genes whose absence would imply the loss of basic biological functions, and that could be typical of the viral family. In view of this, and considering previous reports [1, 19, 22, 23], the amount and identity of baculovirus common genes were reevaluated (Table 2). As a result, P6.9 and Desmoplakin were recognized in this work, as core proteins by using sequence analysis complementary to the standard ones (see Supplementary files available at doi:10.4061/2011/379424).

The group of conserved sequences found in all baculovirus genomes is consistently estimated at about 30 shared genes, regardless of the increasing number of genomes analyzed [22, 148]. Meanwhile, the role or function assigned to several sequences has been renewed, according to new studies. In particular, it has been identified that 38k (Ac98) gene encodes a protein which is part of the capsid structure [121, 122]; P33 (Ac92) is a sulfhydryl oxidase which could be related to the proper production of virions in the infected cell nucleus [123–125]; ODV-EC43 (Ac109) is a structural component which would be involved in BV and ODV generation [126]; P49 (Ac142) is a capsid

Table 1: Baculovirus complete genomes.

Genus	Name	Abbreviation	Code	Accesion number	Genome (bp)	Annotated ORFs	GC%	Ref.
Alphabaculovirus-Group I	*Antheraea pernyi* NPV-Z	AnpeNPV-Z	APN	NC_008035	126629	145	53.5	[27]
	Antheraea pernyi NPV-L2	AnpeNPV-L2	AP2	EF207986	126246	144	53.5	[28]
	Anticarsia gemmatalis MNPV-2D	AgMNPV-2D	AGN	NC_008520	132239	152	44.5	[29]
	Autographa californica MNPV-C6	AcMNPV-C6	ACN	NC_001623	133894	154	40.7	[30]
	Bombyx mori NPV	BmNPV	BMN	NC_001962	128413	137	40.4	[31]
	Bombyx mandarina NPV	BomaNPV	BON	NC_012672	126770	141	40.2	[32]
	Choristoneura fumiferana DEF MNPV	CfDEFMNPV	CDN	NC_005137	131160	149	45.8	[33]
	Choristoneura fumiferana MNPV	CfMNPV	CFN	NC_004778	129593	145	50.1	[34]
	Epiphyas postvittana NPV	EppoNPV	EPN	NC_003083	118584	136	40.7	[35]
	Hyphantria cunea NPV	HycuNPV	HCN	NC_007767	132959	148	45.5	[36]
	Maruca vitrata MNPV	MaviMNPV	MVN	NC_008725	111953	126	38.6	[37]
	Orgyia pseudotsugata MNPV	OpMNPV	OPN	NC_001875	131995	152	55.1	[38]
	Plutella xylostella MNPV	PlxyMNPV	PXN	NC_008349	134417	149	40.7	U
	Rachiplusia ou MNPV	RoMNPV	RON	NC_004323	131526	146	39.1	[39]
Alphabaculovirus-Group II	*Adoxophyes honmai* NPV	AdhoNPV	AHN	NC_004690	113220	125	35.6	[40]
	Adoxophyes orana NPV	AdorNPV	AON	NC_011423	111724	121	35.0	[41]
	Agrotis ipsilon NPV	AgipNPV	AIN	NC_011345	155122	163	48.6	U
	Agrotis segetum NPV	AgseNPV	ASN	NC_007921	147544	153	45.7	[42]
	Apocheima cinerarium NPV	ApciNPV	APO	FJ914221	123876	118	33.4	U
	Chrysodeixis chalcites NPV	ChChNPV	CCN	NC_007151	149622	151	39.0	[43]
	Clanis bilineata NPV	ClbiNPV	CBN	NC_008293	135454	129	37.7	[44]
	Ectropis obliqua NPV	EcobNPV	EON	NC_008586	131204	126	37.6	[45]
	Euproctis pseudoconspersa NPV	EupsNPV	EUN	NC_012639	141291	139	40.4	[46]
	Helicoverpa armigera NPV-C1	HearNPV-C1	HA1	NC_003094	130759	135	38.9	[47]
	Helicoverpa armigera NPV-G4	HearNPV-G4	HA4	NC_002654	131405	135	39.0	[47]
	Helicoverpa armigera MNPV	HearMNPV	HAN	NC_011615	154196	162	40.1	[48]
	Helicoverpa armigera SNPV-NNg1	HearSNPV-NNg1	HAS	NC_011354	132425	143	39.2	[49]
	Helicoverpa zea SNPV	HzSNPV	HZN	NC_003349	130869	139	39.1	U
	Leucania separata NPV-AH1	LeseNPV-AH1	LSN	NC_008348	168041	169	48.6	[50]
	Lymantria dispar MNPV	LdMNPV	LDN	NC_001973	161046	163	57.5	[51]
	Lymantria xylina MNPV	LyxyMNPV	LXN	NC_013953	156344	157	53.5	[52]

Table 1: Continued.

Genus	Name	Abbreviation	Code	Accesion number	Genome (bp)	Annotated ORFs	GC%	Ref.
	Mamestra configurata NPV-90-2	MacoNPV-90-2	MCN	NC_003529	155060	169	41.7	[53]
	Mamestra configurata NPV-90-4	MacoNPV-90-4	MC4	AF539999	153656	168	41.7	[54]
	Mamestra configurata NPV-B	MacoNPV-B	MCB	NC_004117	158482	169	40.0	[55]
	Orgyia leucostigma NPV	OrleNPV	OLN	NC_010276	156179	135	39.9	U
	Spodoptera exigua MNPV	SeMNPV	SEN	NC_002169	135611	142	43.8	U
	Spodoptera frugiperda MNPV-3AP2	SfMNPV-3AP2	SF2	NC_009011	131330	143	40.2	[56]
	Spodoptera frugiperda MNPV-19	SfMNPV-19	SF9	EU258200	132565	141	40.3	[57]
	Spodoptera litura NPV-II	SpliNPV-II	SLN	NC_011616	148634	147	45.0	U
	Spodoptera litura NPV-G2	SpliNPV-G2	SL2	NC_003102	139342	141	42.8	[58]
	Trichoplusia ni SNPV	TnSNPV	TNN	NC_007383	134394	144	39.0	[59]
Betabaculovirus	*Adoxophyes orana* GV	AdorGV	AOG	NC_005038	99657	119	34.5	[60]
	Agrotis segetum GV	AgseGV	ASG	NC_005839	131680	132	37.3	U
	Choristoneura occidentalis GV	ChocGV	COG	NC_008168	104710	116	32.7	[61]
	Cryptophlebia leucotreta GV	CrleGV	CLG	NC_005068	110907	129	32.4	[62]
	Cydia pomonella GV	CpGV	CPG	NC_002816	123500	143	45.3	[63]
	Helicoverpa armigera GV	HearGV	HAG	NC_010240	169794	179	40.8	[64]
	Phthorimea operculella GV	PhopGV	POG	NC_004062	119217	130	35.7	[65]
	Plutella xylostella GV	PlxyGV	PXG	NC_002593	100999	120	40.7	[66]
	Pieris rapae GV	PiraGV	PRG	GQ884143	108592	120	33.2	U
	Pseudaletia unipuncta GV-Hawaiin	PsunGV	PUG	EU678671	176677	183	39.8	U
	Spodoptera litura GV-K1	SpliGV	SLG	NC_009503	124121	136	38.8	[67]
	Xestia c-nigrum GV	XnGV	XCG	NC_002331	178733	181	40.7	[68]
Gamma	*Neodiprion abietis* NPV	NeabNPV	NAN	NC_008252	84264	93	33.4	[69]
	Neodiprion lecontei NPV	NeleNPV	NLN	NC_005906	81755	93	33.3	[70, 71]
	Neodiprion sertifer NPV	NeseNPV	NSN	NC_005905	86462	90	33.8	[71, 72]
Delta	*Culex nigripalpus* NPV	CuniNPV	CNN	NC_003084	108252	109	50.9	[73]

This table contains all of baculoviruses used in bioinformatic studies, sorted by genus (and within them by alphabetical order). MNPV is the abbreviation of multicapsid nucleopolyhedrovirus; NPV is the abbreviation of nucleopolyhedrovirus; SNPV is the abbreviation of single nucleopolyhedrovirus; GV is the abbreviation of granulovirus. The accession numbers are from National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) and correspond to the sequences of complete genomes. Code is an acronym used for practicality. U: unpublished.

protein important in DNA processing, packaging, and capsid morphogenesis [129]; Ac81 interacts with Actin 3 in the cytoplasm but does not appear in BVs or in ODVs [135]; ODV-E18 (Ac143) would mediate BV production [131]; desmoplakin (Ac66) seems to be essential in releasing processes from virogenic stroma to cytoplasm [132]; PIF-4 (Ac96) and PIF-5 (ODV-56, Ac148) are ODV envelope proteins with an essential role in *per os* infection route [145, 147]; Ac68 may be involved in polyhedron morphogenesis [130].

Table 2: Core genes.

	ACN	LDN	CPG	NSN	CNN
Replication					
lef-1 [74]	14	123	74	68	45
lef-2 [74]	6	137	41	57	25
DNA pol [75–78]	65	83	111	28	91
Helicase [79–90]	95	97	90	61	89
Transcription					
lef-4 [91–95]	90	93	95	62	96
lef-8 [91, 96]	50	51	131	81	26
lef-9 [95, 97]	62	64	117	40	59
p47 [91, 98]	40	48	68	49	73
lef-5 [98–101]	99	100	87	58	88
Packaging, assembly, and release					
p6.9 [102–104]	100	101	86	36	23
vp39 [105–108]	89	92	96	89	24
vlf-1 [100, 109–113]	77	86	106	45	18
alk-exo [114–116]	133	157	125	31	53
vp1054 [117]	54	57	138	85	8
vp91/p95 [118]	83	91	101	84	35
gp41 [119, 120]	80	88	104	47	33
38 k [121, 122]	98	99	88	59	87
p33 [123–125]	92	94	93	24	14
odv-ec43 [126–128]	109	107	55	70	69
p49 [129]	142	20	15	63	30
odv-nc42 [130]	68	80	114	41	58
odv-e18 [131]	143	19	14	65	31
desmoplakin [132]	66	82	112	29	92
Cell cycle arrest and/or interaction with host proteins					
odv-e27 [133, 134]	144	18	97	66	32
ac81 [135]	81	89	103	48	106
Oral infectivity					
pif-0/p74 [136–141]	138	27	60	50	74
pif-1 [142–144]	119	155	75	79	29
pif-2 [136, 142]	22	119	48	55	38
pif-3 [142]	115	143	35	69	46
pif-4/19k/odv-e28 [145]	96	98	89	60	90
pif-5/odv-e56 [146, 147]	148	14	18	38	102

The virus names are indicated in three letter code according to established in Table 1.
Numbers in columns indicates the corresponding ORFs of each genome.

The number and identity of shared orthologous genes in every accepted member of each genus were investigated, and the unique sequences typical of each clade as well as those shared between different phylogenetic groups were identified (Figure 2).

This analysis shows that the four accepted baculovirus genera have accumulated a large number of genes during evolution. Probably, many of these sequences have been incorporated into viral genomes prior to diversification processes since they are found in members of different genera. In contrast, other genes are unique to each genus, suggesting that they have been incorporated more recently and after diversification (Table 3). The possibility that non-shared genes found only in one genus which represent baculovirus ancestral sequences deleted in the other lineages should also be considered. In any case, a set of particular genes which could help in an appropriate genus taxonomy of new baculoviruses with partial sequence information were obtained from this analysis.

3. Whole Baculovirus Gene Content

The study of all genes reported in the 57 completely sequenced viral genomes revealed the existence of about

Table 3: Shared genes*.

Core genes
lef-2 (ACN6), lef-1 (ACN14), pif-2 (ACN22), p47 (ACN40), lef-8 (ACN50), vp1054 (ACN54), lef-9 (ACN62), DNA polymerase (ACN65), Desmoplakin (ACN66), ACN68, vlf-1 (ACN77), gp41 (ACN80), ACN81, vp91/p95 (ACN83), vp39 (ACN89), lef-4 (ACN90), p33 (ACN92), helicase (ACN95), 19K (ACN96), 38 K (ACN98), lef-5 (ACN99), p6.9 (ACN100), odv-ec43 (ACN109), PIF-3 (ACN115), pif-1 (ACN119), alkaline exonuclease (ACN133), p74 (ACN138), p49 (ACN142), odv-e18 (ACN143), odv-e27 (ACN144), odv-e56 (ACN148)
Alpha + Beta + Gamma
Polh (ACN8), dbp (ACN25), p48 (ACN103), ACN145, pp34/PEP (ACN131), odv-e25 (ACN94), p40 (ACN101), ACN106/107
Alpha + Beta + Delta
F-protein (ACN23)
Alpha + Beta
pk-1 (ACN10), 38,7 kDa (ACN13), lef-6 (ACN28), pp31/39K (ACN36), ACN38, ACN53, 25K FP (ACN61), LEF-3 (ACN67), ACN75, ACN76, tlp20 (ACN82), p18 (ACN93), P12 (ACN102), ACN108, p24 (ACN129), me53 (ACN139), ACN146, ie-1 (ACN147)
Alpha
orf1629 capsid (ACN9), ACN19, pkip-1 (ACN24), ACN34, ACN51, iap-2 (ACN58/59), ACN104, p87/vp80 (ACN141), ie-0 (ACN71)
Alpha Group I
ptp-1/bvp (ACN1), ACN5, odv-e26 (ACN16), iap-1 (ACN27), ACN30, ACN72, ACN73, ACN114, ACN124, gp64 (ACN128), p25 (ACN132), ie-2 (ACN151)
Beta
CPG4, CPG5, CPG20, CPG23, CPG29, CPG33, CPG39, CPG45, Metalloproteinase (CPG46), CPG62, FGF-1 (CPG76), CPG79, CPG99, CPG100, CPG115, IAP-5 (CPG116), CPG123, CPG135, FGF-3 (CPG140)
Gamma
NSN3, NSN9, NSN11, NSN12, NSN13, NSN16, NSN18, NSN19, NSN20, NSN26, NSN29, NSN34, NSN37, NSN39, NSN42, NSN43, NSN44, NSN51, NSN52, NSN53, NSN54, NSN56, NSN64, NSN72, NSN74, NSN76, NSN77, NSN79, NSN82, NSN85, NSN86, NSN89
Delta
CNN2, CNN3, CNN6, CNN7, CNN9, CNN10, CNN11, CNN12, CNN13, CNN15, CNN16, CNN17, CNN20, CNN21, CNN22, CNN27, CNN28, CNN31, CNN36, CNN37, CNN39, CNN40, CNN41, CNN42, CNN43, CNN44, CNN47, CNN48, CNN49, CNN50, CNN51, CNN52, CNN53, CNN55, CNN56, CNN57, CNN60, CNN61, CNN62, CNN63, CNN64, CNN65, CNN66, CNN67, CNN68, CNN70, CNN71, CNN72, CNN75, CNN76, CNN77, CNN78, CNN79, CNN80, CNN81, CNN82, CNN83, CNN84, CNN85, CNN86, CNN93, CNN94, CNN97, CNN98, CNN99, CNN100, CNN101, CNN103, CNN105, CNN107

*Shared genes are indicated only for one selected specie. See supplementary tables for the respective ORF numbers in each specie.

895 different ORFs, a set of sequences that might be called *the whole baculovirus gene content*. This high number of potential coding sequences contrasts with the range of gene content among the family members, which is between 90–181 genes (*Alphabaculovirus*: 118–169; *Betabaculovirus*: 116–181; *Gammabaculovirus*: 90–93; *Deltabaculovirus*: 109) as well as with the proportion of core genes which represents only 3%. This curious biological feature supports the hypothesis that highlights the great importance of structural mutations in the macroevolution of viruses with large DNA genomes. From this view, the set of genes shared by all members belonging to each baculovirus genus was compared to those corresponding to the *whole genus gene content* (Figure 3).

The analysis shows that Group I alphabaculoviruses and gammabaculoviruses have a lower diversity of gene content with respect to the rest of lineages. This information, coupled with the significant number of genome sequences obtained from Group I alphabaculoviruses, suggests that this lineage of viruses would constitute the newest clade in baculovirus evolution history [149]. This is based on the assumption that Group I alphabaculoviruses have had less time to incorporate new sequences from different sources (host genomes, other viral genomes, bacterial genomes, etc.) since the appearance of their common ancestor.

4. Baculovirus Core Gene Phylogeny

Traditional attempts to infer relationships between baculoviruses were performed by amino acid or nucleotide sequence analyses of single genes encoding proteins such as polyhedrin/granulin (the major component of OBs), the envelope fusion polypeptides known as F protein and GP64, or DNA polymerase protein, among many other examples [149–152].

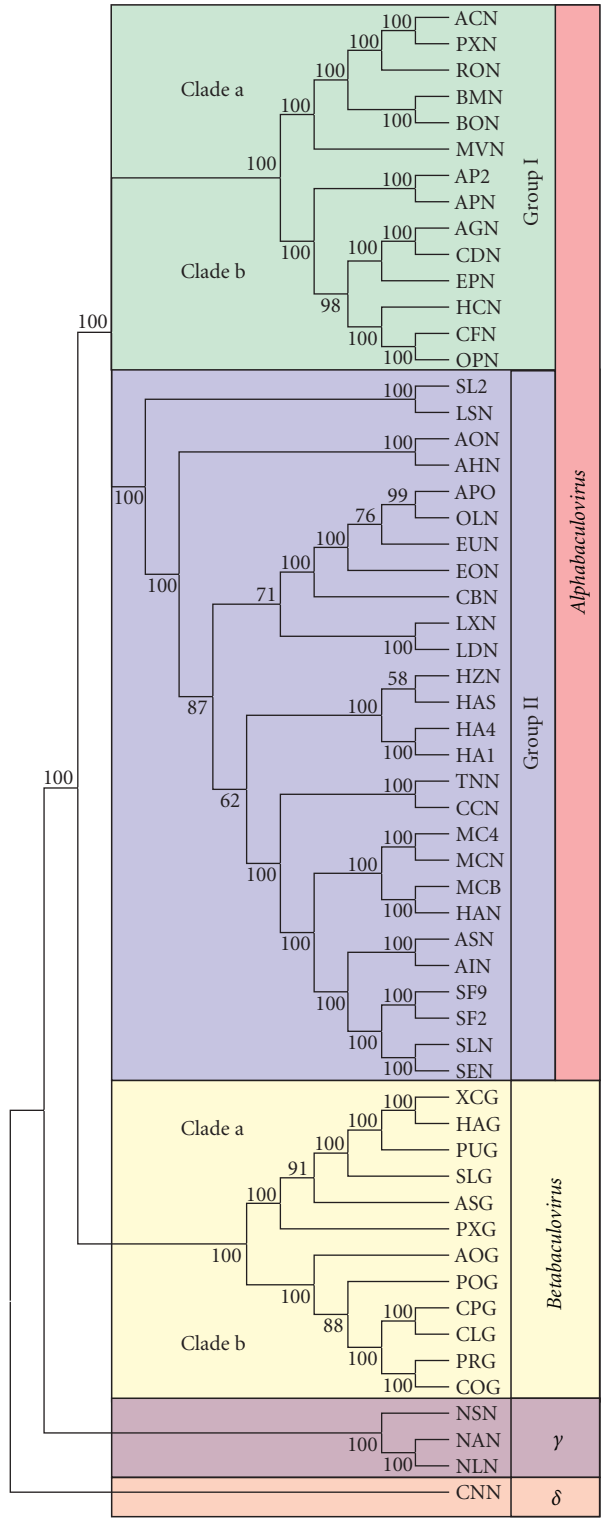

Figure 4: Baculovirus genome phylogeny. Cladogram based on amino acid sequence of core genes. The 31 identified core genes from Baculoviridae family were independently aligned using MEGA 4 [25] program with gap open penalty = 10, gap extension penalty = 1, and dayhoff matrix [26]. Then, a concatemer was generated and phylogeny inferred using the same software (UPGMA; bootstrap with 1000 replicates; gap/missing data = complete deletion; model = amino (dayhoff matrix); patterns among sites = same (homogeneous); rates among sites = different (gamma distributed); gamma parameter = 2.25). Baculoviruses are identified by the acronyms given in Table 1, and the accepted distribution in lineages and genera are also indicated. *Gammabaculovirus* and *Deltabaculovirus* are referenced by Greek letters. The proposed clades of Betabaculoviruses are shown in bold letters.

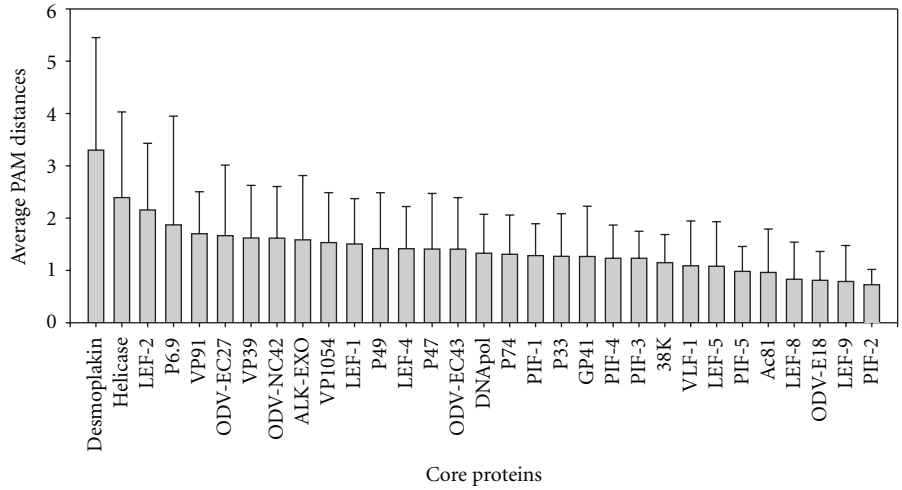

Figure 5: Baculovirus core gene variability. Histograms show the average PAM250 distances for each core gene with their corresponding standard deviations. These values were calculated using MEGA 4 program (UPGMA; bootstrap with 1000 replicates; gap/missing data = complete deletion; model = amino (dayhoff matrix); patterns among sites = same (homogeneous); rates among sites = different (gamma distributed; gamma parameter = 2.25)). PAM (point accepted mutation) matrices refers to the evolutionary distance between pairs of sequences. Given the weak similarity between several core proteins, PAM250 matrix was selected. The divergence considered in this matrix is 250 mutations per 100 amino acid sequence and was calculated to analyze more distantly related sequences. PAM250 is considered a good general matrix for protein similarity search.

Mostly, the evolutionary inferences were in agreement with much stronger subsequent studies based on sequence analyses derived from sets of genes with homologous sequences in all baculoviruses. Thus, these new approaches were based on the construction of common-protein-concatemers which were used to propose evolution patterns for baculoviruses [149].

Then, the fact that a viral family consists of members who share a common pattern of genes and functions and whose proliferation cycle continuously challenges the viral viability turns it essential to take into account their higher or lesser tolerance to the molecular changes. Molecular constraints regarding tolerance to changes in core genes are different from those of other genes. Therefore, core genes should be considered the most ancestral genes which may have diverged in higher or lesser degrees. According to this, a phylogenetic study was performed based on concatemers obtained from multiple alignments of the 31 proteins recognized in this work as core genes for the 57 available baculoviruses with sequenced genomes (Figure 4).

The obtained cladogram reproduces the current baculovirus classification based on 4 genera. Additionally, this approach consistently separates the alphabaculoviruses into two lineages: Group I and Group II. And the same can be observed when analyzing Group I, where the presence of two different clades can be clearly inferred (clade a and clade b). These groupings result in accordance with previous reports [20, 150]. In Group II alphabaculoviruses, a clear clustering may not be identified and would not allow to suggest a subdivision.

In contrast, in the *Betabaculovirus* genus, it is possible to propose their separation into two different clades: clade a (XnGV, HearGV, PsunGV, SpliGV, AgseGV, and PlxyGV), and clade b (AdorGV, PhopGV, CpGV, CrleGV, PiraGV, ChocGV).

Despite the evolutionary inference based on core genes, there was a remaining question: "is the tolerance to changes in all core genes the same?". The answer could be reached by an individual core gene variability analysis for which studies of sequence distance for each baculovirus core gene were performed (Figure 5).

The resulting order of core genes shows that *pif-2* was the most conserved baculovirus ancestral sequence, whereas *desmoplakin* was the gene with evidence of greatest variability. This analysis reveals that genomes can be evolutionarily constrained in different ways depending on the proteins they encode.

The gain of access to new hosts might be an important force for gene evolution. During an infection process, the genome variants that appear with mutations introduced by errors in the replication/reparation machinery could be quickly incorporated into the virus population if the nucleotide changes offered a better biological performance when proteins were translated. The *DNA helicase* gene was considered as an important host range factor being, for this study, the second core sequence showing more variability [87]. However, other sequences like *pif-2* gene would not accumulate mutations because the protein encoded might lose vital functions not necessarily associated with the nature of the host.

5. Conclusions

Baculoviridae is a large family of viruses which infect and kill insect species from different orders. The valuable applications of these viruses in several fields of life sciences encourage their constant study with the goal of

understanding the molecular mechanisms involved in the generation of progeny in the appropriate cells as well as the processes by which they evolve. The establishment of solid bases to recognize their phylogenetic relationships is necessary to facilitate the generation of new knowledge and the development of better methodologies.

In view of this, many researchers have proposed and used different bioinformatic methodologies to identify genes as well as related baculoviruses. Some of them were based on gene sequences [150], gene content [17], or genome rearrangements [152]. In this work, a combination of core gene sequence and gene content analyses were applied to reevaluate Baculoviridae classification. To our knowledge, the most important fact is that this report is the first work which identifies the whole baculovirus gene content and the shared genes that are unique in different genera and subgenera. All this information should be taken into account to group and classify new virus isolates and to propose molecular methodologies to diagnose baculoviruses based on proper gene targets according to gene variability and gene content.

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