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ANNOTATED SEQUENCE RECORD

Genomic sequence analysis of *Helicoverpa armigera* nucleopolyhedrovirus isolated from Australia

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Abstract The complete genomic sequence of *Helicoverpa armigera* nucleopolyhedrovirus from Australia, HearNPV-Au, was determined and analyzed. The HearNPV-Au genome was 130,992 bp in size with a G + C content of 39 mol% and contained 134 predicted open reading frames (ORFs) consisting of more than 150 nucleotides. HearNPV-Au shared 94 ORFs with AcMNPV, HearSNPV-G4 and SeMNPV, and was most closely related to HearSNPV-G4. The nucleotide sequence identity between HearNPV-Au and HearSNPV-G4 genome was 99 %. The major differences were found in homologous regions (*hr*s) and baculovirus repeat ORFs (*bro*) genes. Five *hr*s and two *bro* genes were identified in the HearNPV-Au genome. All of the 134 ORFs identified in HearNPV-Au were also found in HearSNPV-G4, except the homologue of ORF59 (*bro*) in HearSNPV-G4. The sequence data strongly suggested that HearNPV-Au and HearSNPV-G4 belong to the same virus species.

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Introduction

Being pathogenic to many insects, baculoviruses are frequently used as bio-insecticides to control the size of pest populations in nature. It is well established that baculovirus populations exhibit large amounts of genotypic variation, which may have multiple origins, such as geographical or temporal differences, a different host, or even an individual host [1–8]. The cotton bollworm *Helicoverpa armigera*, is a serious global pest that is responsible for economic losses to over 60 cultivated crops and is resistant to chemical insecticides. In China, HearNPV (family *Baculoviridae*, genus *Alphabaculovirus*) has been commercialized and extensively used on cotton fields since 1994 [9, 10]. High levels of genetic variation have also been found within HearNPV populations [6–8]. Now, genomes of five *Helicoverpa* spp. NPVs, including HearSNPV-C1 (China) [11], HearSNPV-G4 (China) [12], HearNPV-NNg1 (Kenya) [13], HzSNPV (USA) [14], and HearMNPV (China) [15], have been sequenced. The gene content and arrangement of HearMNPV were distinct from the other four NPVs, and those four NPV genomes shared very high nucleotide sequence identity except for the homologous regions (*hr*) and the baculovirus repeat ORFs (*bro*). In this study, we sequenced and analyzed the complete genome of another HearNPV, HearNPV-Au, which isolated from Australia.

Materials and methods

The HearNPV-Au used in this study was supplied by Tri-Delta Chemicals Pty Ltd. (Australia) and Henan Jiyuan Baiyun Industry Co., Ltd. (China). Polyhedra of HearNPV-Au were propagated in *H. armigera* larvae and purified by washing with 1 % SDS and distilled water multiple times with centrifugation. The purified polyhedra were solubilized in 0.7 ml alkaline solution (0.1 M Na_2CO_3, 0.1 M NaCl, 0.005 M EDTA, pH 8.0) at 37 °C for 1 h. The pH was adjusted to 7.0 with 0.1 M HCl, 5 µl of 20 mg/ml proteinase K was added and the sample was incubated at 55 °C for 3 h. The genomic DNA was extracted with phenol and chloroform, precipitated with 100 % ethanol, and washed with 70 % ethanol.

A random genomic library of HearNPV-Au was constructed according to the "partial filling-in" method as described previously with minor modifications [16]. Viral DNA fragments ranging from 1.5 to 5.0 kbps were cloned into the *Sal*I site of the pUC19 vector. A total of 464 recombinant plasmids were prepared for sequencing using a BigDye Terminator v3.1 Cycle Sequencing Kit (ABI) on a Genetic Analyzer 3130XL (ABI). The combined sequence generated from these clones represented sixfold genomic coverage. Additional sequences for conformation of ambiguous regions and for filling in of gaps in the assembled sequence were obtained from sequencing of PCR products. All of the sequences were assembled into contigs using SeqMan from the DNASTAR 7.0 software package.

ORFs were defined using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). DNA and protein comparisons were performed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) or Vector NTI Advance Suite v8.0. Multiple sequences were aligned in Clustal X and displayed in GeneDot. Promoter motifs present upstream of the putative ORFs were screened as described previously [17].

Results and discussion

During the assembly of the genome sequence, we found some nucleotide variability. Based on a longest assembled sequence (13.5 kb), the rate of nucleotide variability was 0.19 %. We just picked the predominant nucleotide when nucleotide variability occurred. This confirmed that HearNPV populations exhibit genotypic variation.

The HearNPV-Au genome was 130,992 bp in size (GenBank accession no. JN584482), similar to those of HzSNPV (130,869 bp, GenBank accession no. AF334030) and HearSNPV-G4 (131,405 bp, GenBank accession no. NC002654), with a G + C content of 39 mol%. There were 134 predicted ORFs consist of more than 150 nucleotides. The HearNPV-Au genome shared 94 ORFs with the AcMNPV, HaSNPV-G4 and SeMNPV genomes. Homologues of these 94 ORFs were chosen for the GeneParityPlot analysis. The comparison showed that HearNPV-Au and HearSNPV-G4 were completely co-linear and identical in their gene arrangement (Fig. 1). The comparisons between HearNPV-Au and AcMNPV, SeMNPV were in agreement with results reported previously [12]. A comparison of the locations and predicted amino acid sequences of the 94 ORFs between HearNPV-Au and the other three baculovirus genomes (Table 1) also indicated that HearNPV-Au was most closely related to HearSNPV-G4.

The nucleotide sequence identity between HearNPV-Au and HearSNPV-G4 genomes was 99 %, and the major differences were found in the *hr*s and *bro* genes.

Characterized by the presence of multiple imperfect palindrome sequences, *hr*s may function in gene replication, transcription, recombination and rearrangement events [11, 18–20]. Both the number and location of *hr*s from the

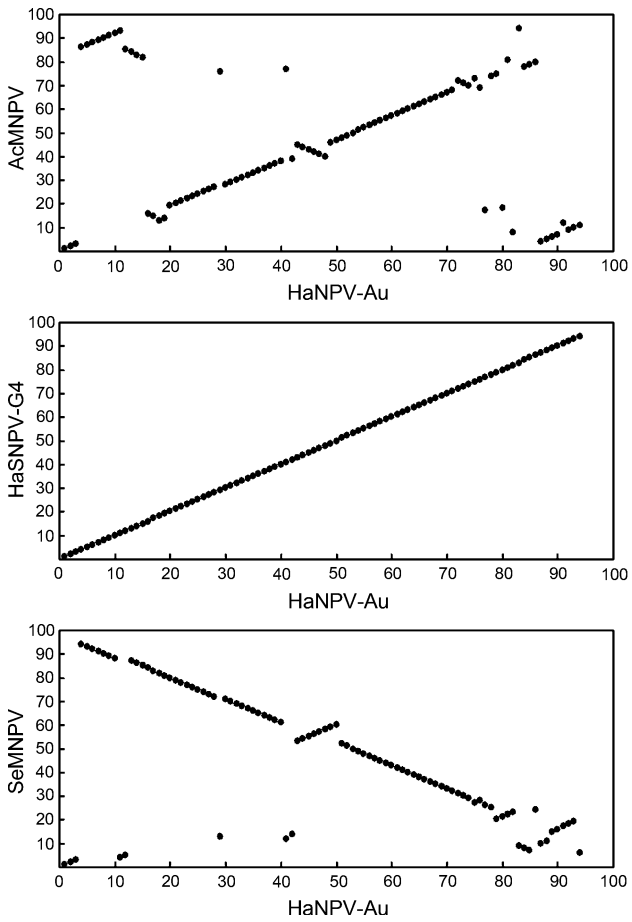

Fig. 1 GeneParityPlot comparison of HearNPV-Au with AcMNPV, HearSNPV-G4 and SeMNPV. Homologous ORFs are plotted based on their relative location in the genome. The horizontal and vertical axes indicate the relative position of each ORF

Table 1 Putative ORFs identified in HearNPV-Au

ORF	Name	Position	Length (aa)	Promoter[a]	Homologous ORFs			Amino acid sequence identity to homologues (%)		
					AcMNPV	HearSNPV-G4	SeMNPV	AcMNPV	HearSNPV-G4	SeMNPV
1	*Polyhedrin*	1 → 741	246	E, L, e	8	1	1	86	100	87
2	*orf1629*	738 ← 1979	413	N	9	2	2	27	99	33
3	*pk-1*	1994 → 2797	267	N	10	3	3	40	99	55
4	*Hoar*	2920 ← 5181	753	E*		4	4		93	28
5		5377 → 5556	59	N		5			97	
6	*Hzorf480*	5724 → 6575	283	E*		6			99	
7		6787 ← 6942	51	N		7			98	
8	*i.e.-0*	6930 → 7787	285	N	141	8	138	32	99	35
9	*p49*	7804 → 9210	468	L	142	9	137	50	99	56
10	*odv-e18*	9221 → 9466	81	L	143	10	136	75	99	60
11	*odv-e27*	9481 → 10335	284	L	144	11	135	50	99	57
12		10381 → 10659	92	L	145	12	134	48	100	58
13		10686 ← 11297	203	N	146	13	133	30	100	32
14	*i.e.-1*	11339 → 13306	655	E*, e	147	14	132	34	99	30
15	*odv-e56*	13359 ← 14423	354	L	148	15	6	51	100	50
16	*me53*	14584 → 15663	359	E*, L	139	16-17	7	24	99	33
17		15666 → 15833	55	L		18			100	
18		15886 ← 16167	93	E*		19			96	
19	*p74*	16188 → 18254	688	N	138	20	131	53	99	55
20	*p10*	18308 ← 18571	87	L	137	21	130	18	100	51
21	*p26*	18654 ← 19457	267	E, L	136	22	129	35	99	43
22		19570 → 19773	67	E*	29	23	128	32	100	48
23	*lef-6*	19849 ← 20412	187	N	28	24	127	32	99	50
24	*Dbp*	20426 ← 21397	323	E	25	25	126	32	100	50
25		21617 → 22018	133	N	26	26	125	42	100	36
	hr1	22019 — 24339								
26		24340 ← 25107	255	E*	34	27	124	37	99	51
27	*ubiquitin*	24947 → 25198	83	L	35	28	123	75	100	78
28		25262 → 25768	168	E*		29			100	
29	*Lese25-like protein*	25788 → 26360	190	L		30			98	
30	*39 k/pp31*	26419 ← 27354	311	N	36	31	120	40	100	33
31	*lef-11*	27320 ← 27703	127	N	37	32	119	39	100	51
32		27672 ← 28388	238	N	38	33	118	52	100	63
33		28620 → 29699	359	E*		34			99	
34	*p47*	29767 ← 31005	412	e	40	35	115	54	99	61
35		31078 → 31749	223	E*	41	36		32	100	
36		31835 → 32077	80	L	43	37	113	30	100	31
37	*lef-8*	32074 ← 34779	901	N	50	38	112	62	99	67
38		34832 → 35410	192	L	51	39	111	31	99	36
39		35551 → 35703	50	L		40			96	
40	*Chitinase*	35711 ← 37480	589	N	126	41	19	66	98	62
41		37524 ← 38066	180	E*	52	42	109	26	100	27
42		38184 → 38594	136	E, L	53	43	108	43	100	56
43		38601 ← 39737	378	e, L		44	107		99	35
44		39745 ← 39972	75	E*, L		45			100	
45	*lef-10*	39932 → 40147	71	N	53a	46	106	38	100	56
46	*vp1054*	40020 → 41075	351	E, e	54	47	105	41	99	49
47		41195 → 41401	68	N	55	48	104	35	100	50
48		41402 → 41596	64	L	56	49	103	37	98	53

Table 1 continued

ORF	Name	Position	Length (aa)	Promoter[a]	Homologous ORFs			Amino acid sequence identity to homologues (%)		
					AcMNPV	HearSNPV-G4	SeMNPV	AcMNPV	HearSNPV-G4	SeMNPV
49		41875 → 42390	171	E, L	57	50	102	42	99	43
50		42441 ← 42923	160	N	59	51	101	46	100	63
51		42935 ← 43201	88	L	60	52	100	42	100	56
52	*Fp*	43413 ← 44066	217	L	61	53	98	63	98	70
53		44238 → 44423	61	E		54			98	
54	*lef-9*	44545 → 46104	519	E	62	55	97	64	99	72
55	*Cathepsin*	46188 ← 47291	367	N	127	56	16	46	98	47
56		47332 ← 47919	195	L		57			99	
57	*gp37*	47990 ← 48829	279	L	64	58	25	60	99	63
	hr2	48830 — 49979								
58	*Bro*	49980 → 51629	549	N		60			96	
	hr3	51630 — 52384								
59	*he65*	52385 → 53095	236	E*	105	61		34	100	
60	*iap-2*	53172 ← 53924	250	E, L	71	62	88	31	99	36
61		53972 ← 54796	274	N	69	63	89	41	99	48
62		54765 ← 55166	133	N	68	64	90	47	99	62
63	*lef-3*	55186 → 56325	379	N	67	65	91	25	99	32
64		56433 ← 58790	785	L	66	66	92	29	99	61
65	*DNA pol*	58821 → 61883	1020	e	65	67	93	46	99	59
66		61960 ← 62418	152	E, L	74	68		26	100	
67	*HzORF384*	62484 ← 62867	127	E, L	75	69	94	23	100	39
68		62873 ← 63130	85	L	76	70	95	40	100	64
69	*vlf-1*	63171 ← 64415	414	L	77	71	82	74	99	67
70		64428 ← 64760	110	L	78	72	81	42	100	43
71	*gp41*	64829 ← 65797	322	E*, L	80	73	80	59	100	59
72		65727 ← 66452	241	N	81	74	79	52	100	66
73		66325 ← 67002	225	e	82	75	78	34	99	45
74	*vp91capsid*	66932 → 69382	816	L	83	76	77	40	99	45
75	*cg30*	69510 ← 70361	283	E*, L	88	77	76	27	100	33
76	*vp39capsid*	70450 ← 71331	293	N	89	78	75	43	100	53
77	*lef-4*	71330 → 72715	461	N	90	79	74	44	99	50
78		72768 ← 73532	254	N	92	80	73	53	100	59
79		73534 → 74022	162	N	93	81	72	55	100	63
80	*odv-e25*	74068 → 74760	230	e, L	94	82	71	42	100	63
81		74792 ← 75289	165	L		83	68		98	31
82	*Helicase*	75308 ← 79069	1253	e, L	95	84	70	42	99	47
83		79026 → 79547	173	N	96	85	69	48	99	63
84		79606 ← 80571	321	N	98	86	67	45	99	53
85	*lef-5*	80467 → 81414	315	N	99	87	66	43	100	51
86	*p6.9*	81408 ← 81737	109	E, L	100	88	65	43	100	67
87		81802 ← 82911	369	L	101	89	64	40	100	51
88		82957 ← 83325	122	E, L	102	90	63	29	100	39
89		83325 ← 84458	377	L	103	91	62	50	99	60
90	*vp80capsid*	84553 → 86370	605	L	104	92	61	27	99	29
91		86367 → 86543	58	N	110	93	60	32	100	64
92		86558 → 87643	361	N	109	94	59	52	100	57
93		87688 → 87972	94	N	108	95	58	47	100	51
94	*odv-e66*	88039 ← 90057	672	L	46	96	57/114	43	99	42/33
95		90078 ← 90908	276	L		97	56		99	60
	hr4	90909 — 93506								

Table 1 continued

ORF	Name	Position	Length (aa)	Promoter[a]	Homologous ORFs			Amino acid sequence identity to homologues (%)		
					AcMNPV	HearSNPV-G4	SeMNPV	AcMNPV	HearSNPV-G4	SeMNPV
96		93508 → 94107	199	E, L	115	98	50	43	100	46
97		94111 → 94467	118	N		99			98	
98	*Parg*	94563 → 96095	510	E, L		100	52		99	27
99		96174 → 96935	253	L	106/107	101	53	47/34	99	57
100		96950 → 97282	110	N		102			100	
101	*iap-3*	97340 ← 98146	268	E*, L		103	110		99	35
102		98143 ← 98298	51	N		104			100	
103	*Bro*	98409 ← 99914	501	L		105			99	
104	*Sod*	100082 → 100561	159	L	31	106	48	75	98	69
105		100568 → 101941	457	e, L		107			99	
106		101994 ← 102572	192	E, e		108			99	
107		102742 → 103098	118	E*		109			100	
108		103109 → 103375	88	L	117	110	47	33	100	37
109		103443 → 105029	528	E	119	111	36	47	99	44
110		105026 → 105262	78	L		112			100	
111	*Fgf*	105285 ← 106190	301	E*	32	113	38	27	100	33
112	*alk-exo*	106318 ← 107604	428	E	133	114	41	41	99	41
113		107624 ← 108013	129	L	19	115	42	30	100	31
	hr5	108013 — 110818								
114		109693 ← 110619	308	E*		115a			100	
115		110820 → 111035	71	E*	111	116		36	100	
116	*lef-2*	111151 ← 111867	238	E*	6	117	12	40	98	45
117	*p24capsid*	112229 → 112975	248	L	129	118	10	32	99	55
118	*gp16*	113037 → 113327	96	L	130	119	9	26	100	31
119	*Calyx/pep*	113379 → 114401	340	e, L	131	120	46	27	99	43
120		114480 → 114944	154	E*	63	121		26	100	
121		115075 → 115665	196	E*, L		122			98	
122	*38.7kd*	115709 ← 116878	389	N	13	123	13	31	99	33
123	*lef-1*	116880 ← 117617	245	N	14	124	14	38	99	47
124		117592 ← 118020	142	E, L		125			92	
125	*Egt*	118165 → 119712	515	E, e, L	15	126	27	44	99	52
126		119912 → 120490	192	N		127			100	
127		120441 → 121241	266	E, L	17	128	29	33	99	30
128		121322 ← 124165	947	L		129	30		99	28
129	*pkip-1*	124571 → 125080	169	N	24	130	32	24	97	39
130	*arif-1*	125147 ← 125944	265	N	21	131	34	31	99	30
131		126205 → 127356	383	L	22	132	35	61	99	66
132		127397 ← 129430	677	E*, L	23	133	8	26	99	39
133		129572 ← 130117	181	E*		134			99	
134		130299 → 130886	195	E*		135			96	

The direction of the ORF in the HearNPV-Au genome is indicated by an arrow

[a] The presence of the following conserved promoter motifs is indicated: E, early promoter motif TATA-box (TATAWAW) within 120 bp upstream region of the initiation codon; W = A/T; number of E, 21. E*, early promoter motif (TATA-box followed by CAKT motif 40 bp downstream) within 120 bp upstream of the initiation codon; K = G/T; number of E*, 26. e, enhancer-like element (CGTGC) within 210 bp upstream of the initiation codon; number of e, 13. L, late promoter motif DTAAG within 120 bp upstream of the initiation codon; D = A/T/G; number of L, 64. N, no TATA-box, enhancer-like element or late promoter motif present within 120 bp upstream region of the ORF; number of N, 41

genomes of HearNPV-Au and HearSNPV-G4 were identical. Two types of repeats, type A and type B, were found in each of the five *hr*s in HearNPV-Au (supplementary material). The sequence identities of the five *hr*s between HearNPV-Au and HearSNPV-G4 were 95.8 %, 99.8 %, 98.9 %, 87.7 %, 99.9 % respectively, which showed that

*hr*4 was more variable. Besides, *hr*1 contained a 58-bp insertion and *hr*4 contained a 289-bp insertion compared with isolate G4, and neither insertion contained a type A or type B repeat. This suggested that *hr*s were less conserved than ORF regions in the *Helicoverpa* spp. NPVs.

Typically with multiple copies per genome, *bro* genes may function in nucleic acid binding, nucleosome association, and nucleocytoplasmic shuttling activity; may influence baculovirus genome diversity; and are involved in recombination between baculovirus genomes [21–25]. HearNPV-Au encodes two *bro* genes, named *bro-a* (ORF58) and *bro-b* (ORF103) based on their gene order. HearSNPV-G4 encodes three *bro* genes, named *bro-a* (ORF59), *bro-b* (ORF60), *bro-c* (ORF105). HearNPV-Au *bro-a*, consisting of 1650 bp, shared 94.7 % nucleotide sequence identity (96 % amino acid identity) with HearSNPV-G4 *bro-b* and contained a 66-bp insertion compared with HearSNPV-G4 *bro-b*. HearNPV-Au *bro-b*, consisting of 1506 bp, shared 99.7 % nucleotide sequence identity (99 % amino acid identity) with HearSNPV-G4 *bro-c*. All 134 ORFs identified in HearNPV-Au were also found in HearSNPV-G4, and HearNPV-Au lacked only the homologue of another *bro* gene, ORF59, in HearSNPV-G4, which was the major reason for the size difference between these two genomes. This might have been caused by genetic variation in recombination, suggesting that *bro* genes might play a role in gene exchange and evolution in different geographic locations.

Conclusions

HearNPV-Au shared 99 % sequence identity with HearNPV-G4, lacking only ORF59 (*bro*). The sequence data strongly suggest that HearNPV-Au and HearSNPV-G4 belong to the same virus species, *Helicoverpa armigera* nucleopolyhedrovirus. Whether these minor differences in the genome affect pathogenicity and host range needs to be determined.

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