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Short Communication

High levels of genetic diversity in *Spodoptera exempta* NPV from Tanzania

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ABSTRACT

The African armyworm, *Spodoptera exempta*, is a major pest in sub-Saharan Africa. A nucleopolyhedrovirus (NPV) is often recorded in later population outbreaks and can cause very high levels of mortality. Research has been addressing whether this NPV can be developed into a strategic biological control agent. As part of this study, the variation in natural populations of NPV is being studied. An isolate of *S. exempta* NPV was cloned *in vivo* and found to contain at least 17 genetically-distinct genotypes. These genotypes varied in size from approximately 115 to 153 kb.

1. Introduction

The African Armyworm, *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) is an episodic migratory pest of the Old World tropics but is most prevalent in sub-Saharan Africa, especially on the eastern half of the continent. During almost annual population outbreaks, *S. exempta* larvae can devastate large areas of rangeland and graminaceous crops (Haggis, 1987) and are capable of achieving densities of 200–1000 larvae per m^2 (Rose et al., 2000; Grzywacz et al., 2008). The limited availability and prohibitive cost of effective chemical control measures means that subsistence farmers can do little to counter this rampant pest in outbreak years (Njuki et al., 2004), although larvae at the end of the outbreak season are often killed in large numbers by an NPV (Rose et al., 2000). The development of *S. exempta* NPV (*Spex*NPV) into a biological control agent may offer a viable control option. *Spex*NPV is a specific and extremely pathogenic natural mortality agent of *S. exempta*, which has shown considerable potential in field spray-trials carried out in northern Tanzania (Grzywacz et al., 2008). To support this work, a basic understanding of the diversity and genetic composition of *Spex*NPV is required. Briefly, we report on the isolation of individual *Spex*NPV genotypes and their genetic characterisation using Restriction Fragment Length Polymorphism (RFLP) profiling. The approximate size of individual genomes is estimated and the phylogenetic relationship between genotypes is also investigated.

2. Materials and methods

The *Spex*NPV isolate was collected in 1972 from *S. exempta* populations in Tanzania, amplified *in vivo* and stored at −20 °C. Restriction endonuclease (REN) analysis of its DNA suggested the presence of multiple genotypes. *Spex*NPV is a multiple nucleopolyhedrovirus (MNPV) that can routinely package numerous genotypes within a single occlusion body (OB). *In vivo* cloning was undertaken to isolate the individual genotypes and was chosen over *in vitro* methodologies to avoid the introduction of artificial selection pressures. Smith and Crook (1988) developed *in vivo* cloning as a simple technique to isolate genotypes from mixed populations of baculoviruses. Slight modifications to their original technique have allowed the successful *in vivo* cloning of genotypes from *Spodoptera exigua* NPV (*Se*NPV) (Muñoz and Caballero, 2000) and *Panolis flammea* NPV (Cory et al., 2005) populations. *In vivo* cloning involves the serial infection of larvae using low viral doses until mortality is assumed to have initiated from a single virus genotype. Individual genetically-distinct isolates are provisionally identified by a lack of sub-molar bands in their REN profiles. The purity of suspected single-genotype isolates can be confirmed through the stability of REN patterns through additional rounds of infection. *In vivo* cloning involved the infection of 600 newly-

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Table 1
Mean size (kb) of EcoRV, BamHI and XhoI fragments of *S. exempta* NPV estimated from a minimum number of three independently run agarose gels.

REN fragments	EcoRV	BamHI	XhoI
A	18.56	17.87	22.50
B	16.01	16.54	21.4
C	14.38	15.2	18.74
D	10.53	14.05	16.21
E	9.57	12.82	7.3
F	8.4	11.53	5.47
G	8.25	10.18	5.08
H	7.96	9.20	4.63
I	7.00	8.53	4.26
J	5.80	8.1	3.43
K	5.25	7.56	3.25
L	5.12	6.58	3.01
M	4.78	5.63	2.96
N	4.34	4.46	2.46
O	3.96	2.98	1.94
P	3.6	2.31	1.83
Q	2.5	1.68	1.03
R	2.36	1.55	0.94
S	2.32		
T	2.20		
U	2.2		
V	2.1		
W	2.07		
X	1.86		
Y	1.67		
Z	1.66		
a	1.33		
b	0.84		
c	0.61		

moulted 3rd instar *S. exempta* larvae in the first round and batches of 35 larvae in subsequent rounds, with a viral dose of 1200 OBs (LD_{10} estimated from Reeson et al., 1998), using the diet-plug contamination method. The *S. exempta* larvae used for cloning came from a culture maintained on a wheatgerm-based semi-synthetic diet at the NERC Centre for Ecology and Hydrology, Oxford. The insects were originally collected from Tanzania in 1996 (Vilaplana et al., 2010). A total mortality of 18% was achieved from the initial round of infection from which 37% of the cadavers were characterised with EcoRV. After two rounds of cloning, 18 genetically distinct single-genotype isolates had been identified, which remained stable through a third *in vivo* passage. The profile of one of the isolates produced sub-molar bands when BamHI and XhoI were introduced into the screen at this point and was therefore discarded. A fourth and final *in vivo* passage of the remaining 17 single genotypes confirmed their stability. The *S. exempta* culture used for *in vivo* cloning was known to support a high level of covert infection (Vilaplana et al., 2010); however, the profiles of the *Spex*NPV clones did not change during passage, indicating that expression of the covert virus was not an issue. A minimum number of three gels per enzyme (XhoI, BamHI and EcoRV) were used to estimate fragment sizes and total genome size (Table 1).

In order to investigate the phylogeny of baculovirus species with no existing sequence information, one approach that has proved successful is to use a concatenated sequence from just a few phylogenetically informative genes (Herniou et al., 2004; Lange et al., 2004; Jehle et al., 2006). For this study, four different genes were selected for their proven phylogenetic potential: (1) the highly-conserved *polh* gene, encoding the OB protein (Zanotto

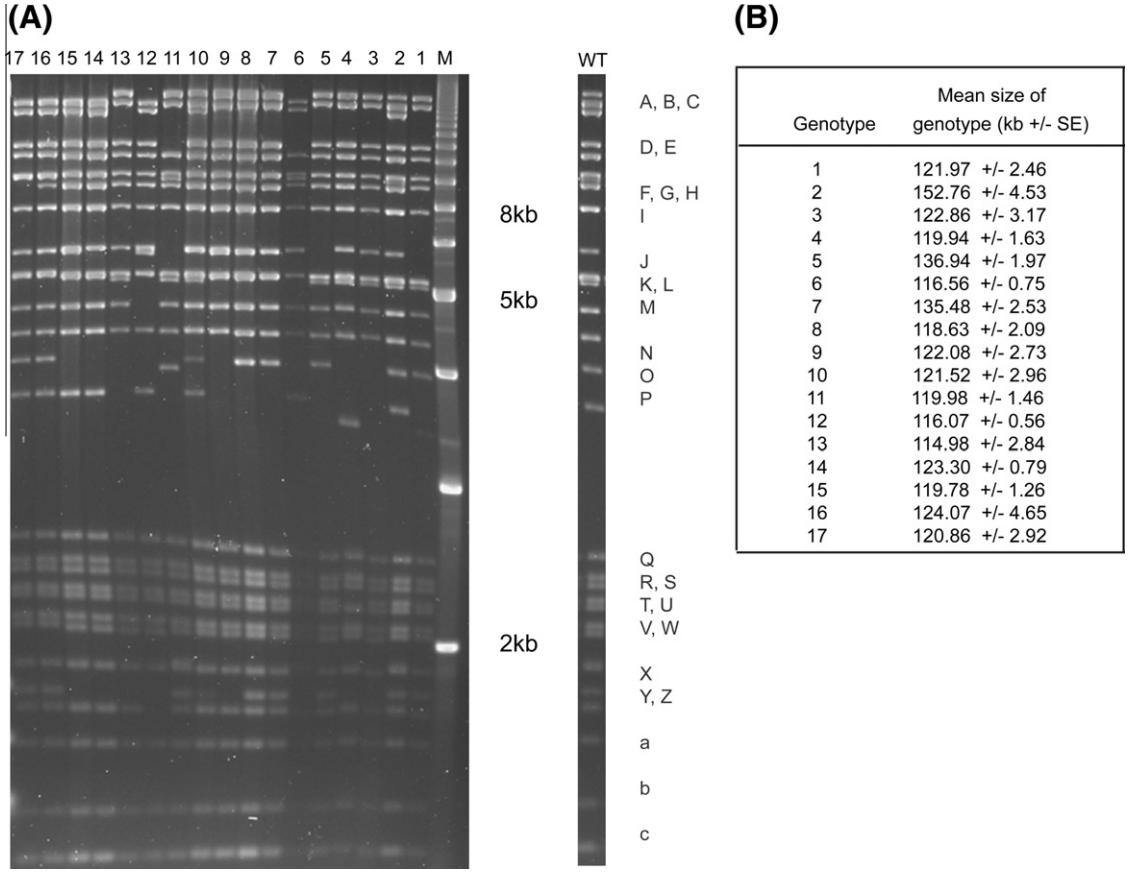

Fig. 1. Restriction endonuclease (REN) analysis used for the estimation of the genome size of *in vivo* cloned *S. exempta* NPV genotypes. (A) EcoRV profiles of 17 genetically distinct, *in vivo* cloned, *Spex*NPV genotypes and wild-type *Spex*NPV fragments, resulting from digestion with EcoRV, named alphabetically, largest to smallest (Vlak and Smith, 1982); (B) mean genome size estimates for the 17 genotypes (± 1 SE) resulting from REN analysis with three different enzymes namely EcoRV, BamHI and XhoI. All fragments sized from a minimum of three separate agarose gels.