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Hierarchical spatial structure of genetically variable nucleopolyhedroviruses infecting cyclic populations of western tent caterpillars

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Abstract

The cyclic population dynamics of western tent caterpillars, *Malacosoma californicum pluviale*, are associated with epizootics of a nucleopolyhedrovirus, McplNPV. Given the dynamic fluctuations in host abundance and levels of viral infection, host resistance and virus virulence might be expected to change during different phases of the cycle. As a first step in determining if McplNPV virulence and population structure change with host density, we used restriction fragment length polymorphism (RFLP) analysis to examine the genetic diversity of McplNPV infecting western tent caterpillar populations at different spatial scales. Thirteen dominant genetic variants were identified in 39 virus isolates (individual larvae) collected from field populations during one year of low host density, and another distinct variant was discovered among nine additional isolates in two subsequent years of declining host density. The distribution of these genetic variants was not random and indicated that the McplNPV population was structured at several spatial levels. A high proportion of the variation could be explained by family grouping, which suggested that isolates collected within a family were more likely to be the same than isolates compared among populations. Additionally, virus variants from within populations (sites) were more likely to be the same than isolates collected from tent caterpillar populations on different islands. This may indicate that there is limited mixing of virus among tent caterpillar families and populations when host population density is low. Thus there is potential for the virus to become locally adapted to western tent caterpillar populations in different sites. However, no dominant genotype was observed at any site. Whether and how selection acts on the genetically diverse nucleopolyhedrovirus populations as host density changes will be investigated over the next cycle of tent caterpillar populations.

Keywords: local adaptation, pathogen variation, restriction fragment length polymorphism, source and sink populations, spatial structure, vertical transmission

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Introduction

The pattern of ecological and evolutionary dynamics in host–parasite populations is determined by variation in resistance within host populations and interactions between host resistance and parasite virulence, as modulated by environmental factors. These interactions are likely to vary among localities, leading to a spatially heterogeneous mixture of host–parasite traits. This process has been described by the geographical mosaic theory of co-evolution, which provides a testable framework for addressing whether and how interacting organisms evolve at different spatial and temporal scales (Thompson 1999). The geographical mosaic theory of co-evolution makes three assumptions; firstly that selection will vary in different locations, secondly that co-evolution will not occur at all locations (there will be hot-spots) and finally

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that co-evolved traits will be mixed by various processes. It would seem likely that such a mosaic structure would be particularly relevant for host–parasite interactions where host populations are spatially structured and in situations where hosts go through large population fluctuations. However, long-term field data on host–parasite interactions, particularly at a landscape scale, are still extremely limited (but see Lively 1999, 2001). To study host–parasite interactions at the landscape level, the patterns of genetic variation of parasites and hosts must be determined.

Nucleopolyhedroviruses (NPVs) are naturally occurring insect pathogens that have primarily been isolated from Lepidoptera. Epizootics of viral disease are often observed during high host densities, and this suggests that these pathogens may play a role in regulating insect dynamics (Anderson & May 1980; Cory *et al*. 1997). Population cycles of forest Lepidoptera provide an excellent arena in which to study the long-term interactions between NPVs and their hosts. In outbreak populations susceptible hosts are numerous and the likelihood of disease transmission is high. However, at low host densities susceptible hosts will be rare and horizontal transmission of virus through larval death and food plant contamination may be limited. In addition, high levels of infection may select for resistant hosts with possible costs (Fuxa & Richter 1989, 1998; Myers & Kukan 1995). In this dynamic interaction between virus and host, we might expect both genotypic and phenotypic variation of virus (and host) to occur over the cycle of host density. Certain traits or genotypes may be at a selective advantage at high host density and others at low. However, this aspect of virus–insect evolution has received little attention in natural field populations. As a first step in exploring the dynamics of variation in insect virus populations, we have undertaken a study of genotypic variation of NPV from 7 populations of western tent caterpillars, *Malacosoma californicum pluviale* (Dyar) (Lepidoptera: Lasiocampidae), at different spatial scales during a period of declining and low host density.

Western tent caterpillars are common defoliators of deciduous trees, particularly red alder, *Alnus rubra*, in northwestern North America. This species has one generation per year with first instar larvae hatching in early to mid-April. Oviposition occurs in mid-summer when each adult female lays her eggs in one egg mass. Western tent caterpillars are gregarious and feed and build silken tents as family groups. Tents provide the larvae with protection from predators and rain and a surface on which to bask in the sun. These conspicuous tents make it possible to assess caterpillar abundance accurately at both low and high densities. Tent caterpillars live as families in tents, as discrete populations of tents on patches of host trees and as populations on individual southern Gulf Islands and areas within the southwestern mainland of British Columbia and south into Washington and Oregon. This island–mainland

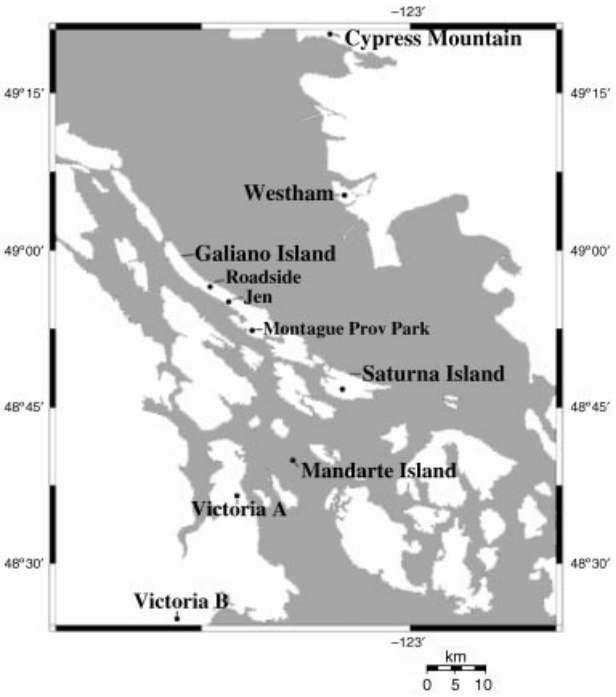

Fig. 1 *Malacosoma californicum pluviale* collection sites in southwestern British Columbia.

distribution allows the simultaneous comparison of virus isolates at several ecological scales. Western tent caterpillar populations have been studied at several locations in southwestern British Columbia since 1976 (Myers 1990, 2000); on the southern Gulf Islands (Galiano, Mandarte and Saturna Islands), on Vancouver Island (Victoria), and on the mainland (Westham 'Island' and Cypress mountain) (Fig. 1). At these locations populations cycle through peak densities every 6–11 years (Myers 2000). NPV is commonly observed in peak populations of western tent caterpillars (Wellington 1964; Myers 1990, 2000; Kukan & Myers 1999). Levels of NPV infection increase with tent caterpillar population density on the southern Gulf Islands and NPV infection is also closely related to density in the mainland populations (Myers 2000). Transmission of NPV within families is not affected by population (family) size but virus transmission between tents increases with family group size (Beisner & Myers 1999).

Analysis of field-collected NPV isolates has shown that baculoviruses can be genotypically highly diverse. Comparison of restriction endonuclease profiles of virus DNA isolated from numerous lepidopteran species has demonstrated that baculovirus isolates can vary between different geographical regions and within single agricultural fields (e.g. Smith & Summers 1978; Gettig & McCarthy 1982; Cherry & Summers 1985; Shapiro *et al*. 1991; Vickers *et al*. 1991; Laitinen *et al*. 1996). However, no studies have addressed genotypic variation in the NPV of the western

tent caterpillar. Several studies have shown that baculovirus isolates from different regions can differ in pathogenicity (e.g. Allaway & Payne 1983; Ebling & Kaupp 1995). Individual genotypes have been cloned from these heterogeneous NPV populations using *in vitro* and, less commonly, *in vivo* cloning techniques (Smith & Crook 1988; Muñoz *et al*. 1999), allowing the phenotypic comparison of individual virus variants (e.g. Lynn *et al*. 1993; Stiles & Himmerich 1998; Muñoz *et al*. 1999). Very recently it has been shown (by *in vivo* techniques) that this variation extends to individual hosts with a single noctuid larva containing up to 24 distinct NPV genotypes (Hodgson *et al*. 2001; Cory & Green submitted for publication). Thus there is considerable potential within the NPV–lepidopteran system for genotypic and phenotypic change at various scales. The persistence of genetic variation in natural NPV populations suggests that heterogeneity is important to virus survival. However, little is known about what generates and maintains this variation (Hodgson *et al*. 2001), and no systematic studies have investigated changes in virus population structure over time, nor its spatial structuring at different ecological levels in field populations.

The current study has two main objectives. The first is to use restriction fragment length polymorphism (RFLP) analysis to describe the genetic variation present in the nucleopolyhedrovirus McplNPV isolated from declining host populations in the field. The second is to use this molecular information to compare virus isolates at different spatial scales to discern geographical patterns or population substructure.

Materials and methods

Collection and rearing of NPV-infected larvae

In the spring of 1998, tent caterpillar larvae were collected from five geographical locations in British Columbia: Cypress Mountain and Westham on the lower mainland and three of the southern Gulf Islands; (Galiano Island, Mandarte Island and Saturna Island) (Fig. 1). Tent caterpillars from each of the locations, including three sites on Galiano Island, were considered to be different populations. In 1998 tent caterpillar populations in all locations were declining (Myers 2000). Up to 30 larvae were collected from each tent (family) (giving between 187 and 726 larvae per population, total 2773 larvae) and from as many tents as possible (between 10 and 37 tents) (Table 1). Family groups were transferred to paper coffee cups and transported to the laboratory where they were fed decontaminated alder leaves (rinsed in 0.5% sodium hypochlorite solution for 60 s followed by distilled water for 60 s). The numbers of larvae killed by NPV, parasitoids and other causes were recorded daily. Time from infection by McplNPV to death is approximately 6–10 days at room temperature (≈ 24 °C) (Frid & Myers 2002) and monitoring was stopped after 10 days of observation to avoid scoring infections arising in the laboratory. NPV infection was diagnosed by the physical characteristics of dead larvae, and where necessary, the presence of occlusion bodies (described as 'OBs' below) in dead larvae was verified by microscopic inspection. Levels of infection ranged from 0% to 50% of tents sampled per site (1% to 13% of individuals per site) (Table 1). All dead individuals were stored at –20 °C. In 1999 and 2000, western tent caterpillar densities were very low and therefore field collections of larvae (and infected individuals) were considerably more limited (0–26 tents per site in 1999 and 0–8 tents per site in 2000; 12 infected individuals in 1999 and nine infected individuals in 2000). However, at one site (Mandarte Island) virus from the same host population was examined over a 3-year period (this was the only site with virus deaths in all 3 years). Larvae were collected and reared as described above.

Table 1 Incidence of nucleopolyhedrovirus infection in western tent caterpillar populations in southwestern British Columbia in 1998–2000

Population	No. of infected tents	Total tents (% inf.)	No. of infected indiv. (%)	Total indiv.
1998				
Galiano Island				
Jen	6	20 (30)	49 (13)	377
Montague	5	10 (50)	20 (11)	187
Roadside	4	12 (33)	30 (13)	225
Saturna Island	6	25 (24)	47 (10)	488
Mandarte Island	3	22 (14)	25 (6)	414
Westham	0	37 (0)	0 (0)	726
Cypress	0	23 (0)	0 (0)	356
1999				
Galiano Island				
Jen	0	2 (0)	0	8
Montague		0		0
Roadside	0	2 (0)	0	29
Saturna Island	0	5 (0)	0	100
Mandarte Island	1	6 (17)	11 (21)	53
Westham	1	26 (4)	1 (0.002)	597
Cypress	0	1 (0)	0	26
2000				
Galiano Island				
Jen	0	1 (0)	0	17
Montague		0		0
Roadside	2	2 (100)	3 (8)	37
Saturna Island	0	1 (0)	0	17
Mandarte Island	2	8 (25)	3 (4)	68
Westham	3	4 (75)	5 (6)	77

inf., infected; indiv., individuals.

Extraction of McplNPV DNA from infected larvae

Virus was extracted from individual caterpillars by macerating the larva with distilled water in an Eppendorf tube. 'OBs' were separated from insect debris with a series of three washes and low speed centrifuge spins (63 *g* for 30 s). They were then pelleted with a high-speed centrifuge spin (12 100 *g* for 20 min) and re-suspended in 1 mL of distilled water. Aliquots of 'OBs' were stored at –20 °C for DNA analysis. Virions were released from the polyhedra by treatment with an alkali lysis solution (1 M Na_2CO_3, 150 mM NaCl, 0.01 mM ethylenediaminetetraacetic acid, pH 10.8) at 37 °C for 60 min and then pelleted at 12 100 *g* for 30 min. The pellet was re-suspended in 20 μL of proteinase K (20 mg/mL) plus 500 μL of proteinase K buffer (10 mM Tris–HCl pH 7.4, 10 mM ethylenediaminetetraacetic acid, 150 mM NaCl, 0.4% sodium dodecyl sulphate) and incubated at 37 °C overnight. Use of a standard phenol : chloroform protocol often resulted in DNA degradation, thus an alternative technique was developed using Puregene™ (Centra System Inc.) to remove proteins. Subsequently DNA was precipitated with sodium acetate (3 M, pH 8.0) and 100% ethanol at –20 °C for 3 h, then re-pelleted at 12 100 *g* for 30 min. Following a wash with 70% ethanol, DNA was re-pelleted and re-suspended in 65 μL of TE buffer at room temperature and then stored at 4 °C.

Characterization of McplNPV genome

Viral DNA (≈ 2 μg, estimated from agarose gels) was digested with *Hin*dIII, *Eco*RI and *Xho*I (Gibco BRL) for 12 h at 37 °C using the manufacturer's recommended conditions. A subset of 13 samples of NPV from field-collected larvae was digested with a combination of the three enzymes to determine the size of the genome. Ten samples were digested twice to ensure restriction patterns were repeatable. Restriction fragments were run on 0.7% agarose gels for 24 h at 65 V in 1 × TBE. Both gel and running buffer contained ethidium bromide at a final concentration of 0.5%. DNA fragment size was estimated by comparison with λ-*Hin*dIII molecular marker (NEB Biolabs).

Genotypic variation in McplNPV isolates

The extent of genotypic variation present in each McplNPV isolate (i.e. the virus extracted from a single larva) was examined using *Hin*dIII only because digestion with *Eco*RI and *Xho*I was frequently unsuccessful. Restriction endonuclease profiles were compared to the profile of the most common genotype. New fragments falling between two previously labelled fragments were given a letter/number combination. Each unique fragment pattern was considered a new genetic variant.

F-*statistics*

The frequency of virus variants at three spatial scales was used to examine virus population structure. The genetic variation of each McplNPV population was quantified by using the distribution and frequency of each genetic variant to test the null hypothesis that genetic variants were randomly distributed. Analysis of molecular variance, AMOVA (ARLEQUIN version 1.1, Schneider *et al*. 1999) was used to produce estimates of the variance components and F-statistics associated with each hierarchical level tested. The analysis generated three *F* statistics; the first, F_{CT}, is an index of the variant frequencies among populations, the second, F_{SC}, is an index of the variant frequencies among family groups within each population, and the third, F_{ST}, is the proportion of total variation among families. *F*-values not significantly different from zero were interpreted as the random distribution of genetic variants across the study region. All tests of significance were conducted at $\alpha = 0.05$.

The *F*-statistics calculated here are used only as an index of the distribution of variant frequencies among the various groups tested. These data are not estimates of nucleotide divergence, and therefore provide no information concerning the relatedness of individual genetic variants. A caveat of using the AMOVA for this system is that *F*-statistics are interpreted under the assumption that restriction analysis is performed on nonrecombining DNA. Here, RFLP analysis was performed on total baculovirus DNA, which is subject to recombination (e.g. Crozier *et al*. 1988; Martin & Weber 1997). Although recombination is thought to be common in baculoviruses, most data have been obtained from *in vitro* studies, and the rates of recombination in wild populations are not known nor are their effects on variant diversity. Given that the production of certain genetic variants may be strongly influenced by recombination, the detection of population structure in the current analysis was interpreted as the likelihood of sampling a given genetic variant from a given population. Population differentiation is therefore based solely on the presence or absence of individual virus variants.

Results

Incidence of NPV infection

McplNPV infection was found in caterpillars from all the Gulf Island populations in 1998 (Table 1), whereas no virus infection was found in the mainland populations. Galiano Island (Montague site) had the highest level of NPV infection (50%) (as measured by the proportion of infected families), followed by Saturna Island (24%) and then Mandarte Island (14%) (Table 1). If NPV infection is assessed in terms of individuals infected, infection levels

Table 2 Estimated sizes (kbp) of restriction fragments of the McplNPV genome cut with *Hin*dIII, *Eco*RI and *Xho*I

Band	*Hin*dIII	*Eco*RI	*Xho*I
A	18.9	18.5	18.8
B	14.5	18.0	18.1
C	12.1	14.4	16.7
D	10.7	12.9	14.4
E	7.7	11.0	10.9
F	6.1	9.6	9.0
G	5.7	8.2	8.5
H	4.3	7.8	8.2
I	4.1	7.4	6.0
J	3.3	6.0	3.7
K	3.2	5.5	1.7
L	3.0	5.2	0.94
M	2.8	4.3	0.92
N	2.4	3.2	
O	1.0	3.0	
P	1.0	1.6	
Q		1.5	
Total size	100.8 ± 0.12	122.5 ± 0.13	118.0*

Fragment sizes and genome sizes are the mean size of 10 restriction endonuclease profiles (viral isolates) from two to six gels.
*Estimated from four profiles.

did not rise above 13% in any site in 1998 (Table 1). In 1999 the number of tent caterpillars declined markedly at all sites and only two virus-infected family groups were found, one on Mandarte Island and the other at Westham (Table 1). In 2000 the number of families with infected individuals increased slightly to six (Table 1).

Virus characterization

The McplNPV genome is approximately 122.5 ± 0.13 kilobases (kb) (Table 2). Only bands found in a majority of isolates were sized. Lettering is thus the same as for McplNPV Variant 10 (see below, Fig. 2). It should be noted that this approximation may be an underestimate because of difficulties in detecting fragments smaller than 1 kb.

Genetic variation in NPV isolates

A total of 13 distinct genetic variants was found in the 39 virus isolates (dominant genotype isolated from a single larva) collected from western tent caterpillars in the field in 1998 (Fig. 2). The most common genetic variant was V10 [found in six isolates within one site (island)], V8 (found in five isolates from two populations on two separate islands), V13 (found in two sites on one island) and V12 (found in five isolates from one population), while the least common variants were V2, V5 and V6, found in one isolate (larva) each. Fragments common to all isolates were A, B, C, D, E, F, I, J, L, M, N, O and P (Fig. 2). Three bands were lost or showed size changes in some variants, G (5.7 kb), H (4.3 kb) and K (3.2 kb). Additional bands were found between the positions of the original G and H bands and between the I and J bands, however, further analysis would be needed to account for the observed changes.

Hierarchical analysis of Hin*dIII virus variants*

Variation was detected both within and between populations (sites). Although the sample sizes were small, different variants were predominant in each population, all populations generated more than one variant and all populations, except for Mandarte Island, had at least one unique variant (Table 3). The distribution of *Hin*dIII virus variants was examined by pooling sample and site data into three different hierarchical arrangements. AMOVAS were used to compare the variant frequencies among these populations. This was accomplished by designating each host population as a group and family groups within each

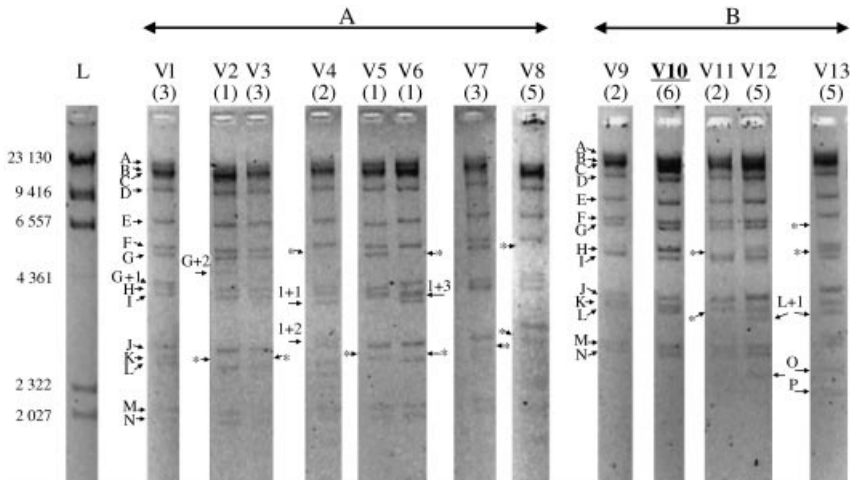

Fig. 2 *Hin*dIII restriction endonuclease fragment profiles of 13 unique variants of McplNPV DNA isolated from 39 *Malacosoma californicum pluviale* larvae collected in the field in 1998. Variants have been assigned numbers V1 to V13. The number in parenthesis below each variant represents the frequency of isolates sharing the same DNA profile. V10 was the predominant genotype and all other isolates are compared with it. Asterisk indicates the absence of bands within each profile as compared to V10. New fragments are indicated by arrows and assigned a new letter/number combination. L is the λ-*Hin*dIII molecular weight marker; size in kilobase pairs (kbp). A and B are two separate agarose gels.

Table 3 Frequencies of *Hin*dIII genetic variants of McplNPV isolated from individual *Malacosoma californicum pluviale* larvae collected in the field in 1998

Collection site	No. of families from which data were collected	Family	*Hin*dIII variant (no. of isolates sharing the same profile)
Saturna Island	5	A	V1 (1), V10 (1)
		B	V10 (3)
		C	V10 (1)
		D	V9 (1)
		E	V1 (1), V10 (1)
Mandarte Island	2	A	V8 (4)
		B	V9 (1)
Galiano Island			
Montague Provincial Park	2	A	V5 (1)
		B	V6 (1), V8 (1), V13 (3)
Jen	3	A	V7 (3), V11 (2), V12 (4)
		B	V12 (1)
		C	V13 (2)
Roadside	3	A	V1 (1), V2 (1), V3 (1), V4 (1)
		B	V3 (2)
		C	V4 (1)

McplNPV variants are labelled V1 to V13; individual families are labelled A to E within each site.

host population as subpopulations. The F_{CT} produced by this grouping, 0.246, was statistically different from zero ($P < 0.001$) suggesting that the distribution of genetic variants was not random, and that variants of McplNPV in southwestern British Columbia were structured at the population level. The comparisons among families within populations (F_{SC}) 0.262, also statistically different from zero ($P < 0.025$), suggest that the distribution of genetic variants among family groups was not random. The F_{ST} of 0.444 ($P < 0.001$; proportion of total variation among family groups) indicates that a high proportion of the variation can be explained by family groupings and suggests that genetic variants within family groups are often more similar than those among populations. However, of the nine families for which isolates were obtained from more than one individual, five had more than one variant.

Comparisons between years

A small number of Mandarte Island samples allowed for the comparison of viral isolates from the same population over a 3-year period (1998–2000). Restriction fragment patterns for the 1998, 1999 and 2000 Mandarte Island isolates are shown in Fig. 3. Host densities were low and infection was limited to three families in 1998 (although material from only two families produced restriction fragment patterns), only one family in 1999 and two families in 2000 (although restriction fragment patterns were only obtained from one family). Within each family group the NPV variants were identical, whereas there were small differences among families in the three years with V8 and V9 occurring in 1998, V14 in 1999 (new variant) and V3 in 2000.

Samples collected during the spring of 2000 from Galiano Island (three individuals) and Westham (one individual) were also used to assess the amount of variation present within the viral populations at very low host densities and restriction endonuclease patterns are shown in Fig. 4. All Galiano Island isolates are identical to V3 from the 1998 Galiano (Roadside) samples and the isolate found on Mandarte in 2000. The Westham isolate is identical to V10 found on Saturna Island in 1998. This suggests that there is only limited variation between relatively isolated McplNPV populations at very low host densities.

Discussion

This study shows that considerable genetic variation is present within the *Malacosoma californicum pluviale* NPV wild-type populations, and that this variation is structured at different spatial scales: this is the first study to analyse baculovirus populations in the field in such detail. Fourteen dominant genetic variants were identified in 48 McplNPV isolates from individual host larvae examined across 3 years of declining and low-density host populations. Use of further restriction enzymes may have increased the number of variants found but the DNA from western tent caterpillar NPV did not prove to be easy to examine using restriction endonucleases and cutting was frequently not successful. Western tent caterpillars are

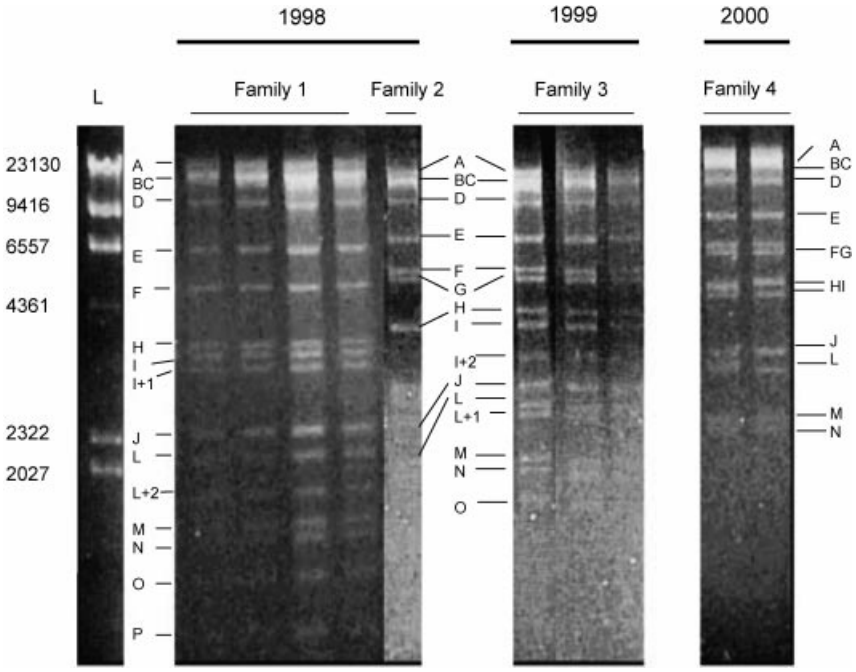

Fig. 3 *Hin*dIII restriction endonuclease profiles of McplNPV DNA from western tent caterpillars collected from Mandarte Island in 1998, 1999 and 2000. Insects which originated from the same family are indicated. Band labelling as in Fig. 2. L is the λ-*Hin*dIII molecular weight marker, sizes in kbp.

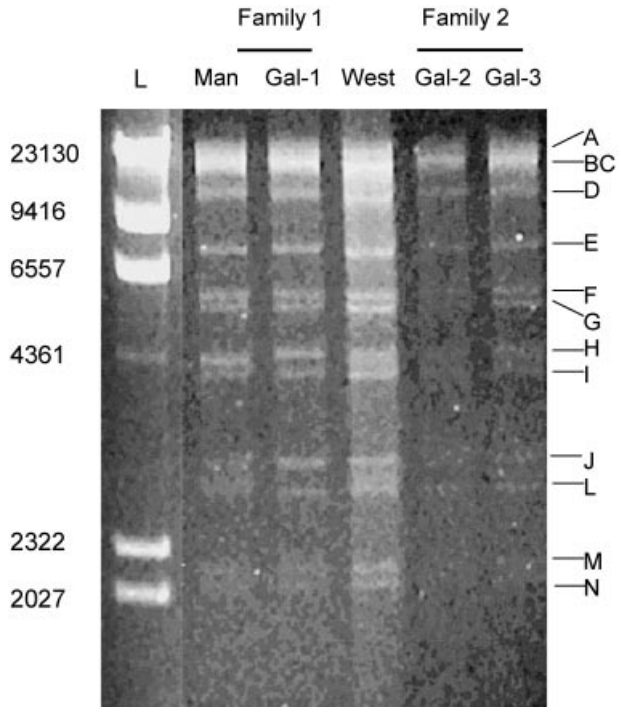

Fig. 4 *Hin*dIII restriction endonuclease profiles of McplNPV DNA from western tent caterpillars collected from Mandarte Island (Man), Westham (West) and Galiano Island (Gal) (roadside site) in 2000. Band labelling as in Fig. 2. L is the λ-*Hin*dIII molecular weight marker, sizes in kbp.

gregarious, with family groups initiated from single egg batches forming silken tents. We thus hypothesized that virus isolates collected from larvae within these families would be the same (and also more similar although the analysis does not test for this). The hierarchical analysis provided strong evidence that larvae from within a family group tend to be killed by the same virus variant, although in more than half of the families in which multiple larvae were examined, more than one genotypic variant was found. In British Columbia, western tent caterpillars are found on isolated patches of suitable host trees, often themselves on separate islands. Again the analysis provided evidence that this isolation leads to some degree of differentiation of the viruses that infect individual caterpillars at the population (site) level.

There are several caveats when interpreting these data. First, sample sizes were limited, although host densities were low, and therefore samples represent a significant proportion of the population. Second, the *F*-statistics used to measure the structure of variation in viral isolates do not reflect the relatedness of individual genetic variants, and therefore, cannot be used to determine the relatedness of virus populations. Estimates of nucleotide divergence would provide more information about the genetic relatedness of the variants between populations.

Genetic differentiation of NPV at the different ecological levels may result from selection among different variants within populations, chance differences in genotypic frequencies among the founders of each population, genetic drift, or any combination of the above. It is also possible that new viral variants are constantly being generated to create similar genotypes. Recombination, point mutations, DNA insertions and deletions, and acquisition of host DNA are common in baculovirus genomes (Crozier *et al*. 1988; Crozier & Ribeiro 1992; Martin & Weber 1997). However, the extent to which each of these factors contributes to the

variability of viruses in nature is unknown (Crozier & Ribeiro 1992). Furthermore, more detailed study in both the laboratory and in the field will be necessary to begin to differentiate among these processes. The distribution of some of the virus variants in different sites might suggest that either McplNPV isolates can be moved between sites with adult moths and then transmitted vertically to offspring (Kukan 1999), or that the virus is dispersed by birds or wind (e.g. Entwistle *et al*. 1993). However, at low host population densities it is less likely that significant immigration among sites, particularly those on different islands, would occur.

The occurrence of genetic variation in natural baculovirus isolates is well documented (e.g. Lee & Miller 1978; Smith & Summers 1978; Gettig & McCarthy 1982; Kislev & Edelman 1982; Maeda *et al*. 1990). However, the role of this variation is not understood and few attempts have been made to tie genetic variation in the virus to host ecology and dynamics. In many instances genetic variation has only been studied by looking at mixed larval isolates collected in one or several sites, although some studies have documented baculovirus variation in a more systematic manner. Shapiro *et al*. (1991), studying *Spodoptera frugiperda* (SfNPV), used RFLP analysis and nucleotide divergence to compare 22 SfNPV isolates over a large ecological scale [15 isolates within Louisiana to seven isolates outside Louisiana (Georgia, Colombia, Venezuela, Mexico)] as well as over a smaller scale (isolates within single agricultural fields). Isolates from within Louisiana were found to be more similar than those outside Louisiana. Significant SfNPV genetic variation was also found within single agricultural fields, though variation within agricultural fields was not structured. Studies in forest insects are rare although variation in the Douglas-fir tussock moth (*Orgyia pseudotsugata*) NPV has been investigated in 10 sites in British Columbia (Laitinen *et al*. 1996). Eight genotypes were found, two of these were abundant/common but their frequency of occurrence varied with site. Multiple genotypes were usually found within individual sites (Laitinen *et al*. 1996). Temporal and spatial variation has been studied by Gelernter & Federici (1990) who investigated *Spodoptera exigua* an agricultural pest, and its NPV (SeNPV), during viral epizootics over 3 years in a large region of California. They concluded that a single dominant genotype was responsible for all the epizootics monitored, although variants of this genotype were also collected. Similarly in Spain, the same RFLP variants of SeNPV were found in natural populations of *S. exigua* over several years (Caballero *et al*. 1992). However, in several of these examples, although the original virus was collected from single host larvae, each was amplified before analysis and this may have resulted in a loss of information and changes in genotypic structure.

As populations of tent caterpillars increase, interactions among larvae from different family groups will also increase as larvae move following local defoliation of tree branches (Beisner & Myers 1999). At high densities large groups of caterpillars often form at the tops of trees and movement and aggregation of larvae potentially facilitates the spread of virus. Among hypotheses on how virus diversity might vary with high host density, two very different hypotheses can be generated. First, if one variant is particularly virulent it might come to dominate during an epizootic (Gelernter & Federici 1990). Thus we would predict reduced variation with increasing host density. On the other hand, virus diversity might increase as the overall production of virus accelerates within and among host populations and opportunities for different viral genotypes to be expressed increase.

When host population density is low and tents are dispersed, virus transmission is primarily among members of the same family and these are likely to be infected by the same virus variant. We have observed lower virus diversity within families than between families at low host density. We have not yet evaluated this variation at high host density. When host and pathogen densities are high, larvae may ingest virus from multiple cadavers and become infected by a number of 'OBs' which themselves could contain diverse genetic variants. Multiple sites of infection can occur in the insect midgut so that many variants can infect separately, or alternatively, new genotypes could be generated via recombination between co-infecting virus genotypes.

To gain an insight into the role and importance of genotypic variation in baculoviruses, links must be made to changes in phenotype. Mixed genotype baculovirus isolates from different geographical regions have been shown to vary in biological activity (e.g. Hatfield & Entwistle 1988; Ebling & Kaupp 1995), as have virus clones (e.g. Lynn *et al*. 1993; Ribiero *et al*. 1997; Stiles & Himmerich. 1998; Hodgson *et al*. 2001), but phenotypic differences between virus originating from individual larvae have received less attention. From an ecological perspective the larva perhaps represents the most relevant unit of virus inoculum. The next step is to establish whether the genotypic diversity seen in the tent caterpillar NPV populations indicates ecologically relevant variation. The variation in DNA profiles of NPV collected in multiple sites and over several years (on Mandarte Island) indicates that a single genotype is not characteristic of low-density populations, although they may have equivalent phenotypes. However, additional factors are likely to influence the generation and maintenance of pathogen variation, including changes in the resistance of the host, host density, environmental factors and other more local components of the host's ecology, such as food plant (Hodgson *et al*. 2002). Understanding the basis and dynamics of baculovirus variation in natural populations will give us an insight into the evolution of pathogens and their hosts in general and baculoviruses

in particular. It may also aid in the development of more realistic evolutionary and population dynamics models of host–pathogen interaction. Whether and how selection acts on the genetically diverse virus as host density changes will be investigated over the next cycle of tent caterpillar populations.

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