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Nucleopolyhedroviruses of forest and western tent caterpillars: cross-infectivity and evidence for activation of latent virus in high-density field populations

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Abstract. 1. Cyclic population dynamics of forest caterpillars are often associated with epizootics of nucleopolyhedrovirus, but it is not known how these viruses persist between generations or through the fluctuations in host population density.

2. To explore the question of virus persistence at different phases of the population cycle, the nucleopolyhedroviruses of two species of tent caterpillar that co-occur in British Columbia, Canada, *Malacosoma californicum pluviale* (western tent caterpillar) and *Malacosoma disstria* (forest tent caterpillar), were characterised. The cross-infectivity of the viruses in these two host species was investigated to determine whether there might be a route for virus persistence via the alternative host species. Any virus produced in the cross-infections was characterised to confirm true cross-infection or to ascertain whether cross-inoculation triggered latent virus persisting within the population.

3. The virus associated with forest tent caterpillars (MadiNPV) did not infect western tent caterpillars from low-density populations, nor did it trigger a latent virus infection; however, inoculation of forest tent caterpillars from high-density populations with virus from western tent caterpillars (McplNPV) resulted in viral infection, but without a dose–response relationship.

4. Analysis of DNA profiles of virus resulting from cross-infection of the forest tent caterpillar with McplNPV, revealed that 88% of these infections were caused by MadiNPV rather than McplNPV; however the virus from all 44 infected individuals was identical and differed in DNA profile from the stock MadiNPV used for cross-infection. This suggests strongly that forest tent caterpillars from high-density field populations harbour a latent, persistent, or sublethal form of MadiNPV that was triggered by exposure to nucleopolyhedrovirus from the western tent caterpillar.

5. Virus was not activated in western tent caterpillars collected over 2 years of late population decline and the first year of population increase.

Key words. Baculovirus, cross-infectivity, environmental stressors, latency, persistence, population fluctuation, sublethal infection, tent caterpillar, vertical transmission.

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Introduction

The causes of cycles in animal populations have long been a major focus for ecological research, however only relatively recently has the role of disease in animal population cycles been considered and studied in field populations. Fluctuating populations of several species of forest Lepidoptera are associated with epizootics of nucleopolyhedrovirus (Baculoviridae) (Myers, 1988). Nucleopolyhedroviruses are DNA viruses that are unusual in that they are primarily transmitted horizontally via occlusion bodies that are released after the death of infected individuals (Cory *et al*., 1997). These viruses are considered to be potentially important factors affecting the dynamics of insect populations because of their association with high host numbers (Anderson, & May 1981); however empirical field studies on the long-term interaction between baculoviruses and their hosts are rare. Western and forest tent caterpillar, *Malacosoma californicum pluviale* (Dyar) and *Malacosoma disstria* (Hübner) (Lepidoptera: Lasiocampidae), populations are among the best-studied species of forest caterpillar in terms of population and virus dynamics (Wellington, 1962; Kukan & Myers, 1997, 1999; Myers, 2000). Although the ranges of these caterpillars overlap (Myers, 1993), simultaneous outbreaks of the two species occur rarely and have not been studied. In British Columbia, the population cycles of these two species have been largely out of phase with recent peak densities of western tent caterpillars in south-western British Columbia in 1986–1987 and 1996–1997, and of forest tent caterpillars in north-central British Columbia in 1992–1993 and 1996–2000 (Myers, 2000; J. H. Myers, pers. obs.).

Although horizontal transmission is likely to be the main route for virus transmission in high-density host populations, it does not explain how the viruses persist when host population densities are low. Nucleopolyhedrovirus occlusion bodies can persist for months or even years when protected from ultraviolet irradiation, however these virus reservoirs are only likely to occur in protected sites such as the soil or crevices in the bark (e.g. Weseloh & Andreadis, 1986; Carruthers *et al*., 1988) that later generations of the insect may never encounter. Baculoviruses are known to be transmitted vertically from adults to offspring (Kukan, 1999). Additionally, survivors of baculovirus challenge can exhibit a range of sublethal effects, including reduced fecundity (e.g. Rothman & Myers, 1996; Myers *et al*., 2000). Both vertically transmitted virus and sublethal effects could theoretically have an important impact on host–pathogen dynamics (Anderson & May, 1981), and a growing body of evidence suggests that sublethal nucleopolyhedrovirus infection may influence the population dynamics of tent caterpillars (Rothman & Myers, 1994; Myers & Kukan, 1995; Myers, 2000). In particular, the reduced fecundity of declining populations may be explained by sublethal infection. Larvae infected shortly before pupation may survive but show the cost of surviving infection in terms of reduced fecundity of adults; however a link between sublethal effects and sublethal levels of infection has yet to be demonstrated. A particular area of debate has been over the nature of the virus during vertical transmission; there is evidence that virus can be transmitted on the surface of the egg and cause overt disease, however there has also been considerable speculation about the existence of latent baculovirus infections (Cory *et al*., 1997). Numerous triggers or stress factors that convert a latent to an overt infection have been discussed over the last 100 years (see Podgwaite & Mazzone, 1986, for a review), however it is only with the more recently available molecular techniques that it has become possible to clearly characterise virus isolates, confirm inoculum purity, and detect low levels of virus infection.

The interaction of the western tent caterpillar and the forest tent caterpillar nucleopolyhedroviruses was investigated as part of a long-term study of the role of nucleopolyhedrovirus in tent caterpillar dynamics. If they are cross-infective, epizootics of virus in one species could influence the population dynamics of the other, and one species might maintain a virus reservoir for the other species. If the viruses are not cross-infective, cross-inoculation could still exert an influence by triggering any latent virus being carried by field populations. Although a range of factors has been implicated in the triggering of latent infection, only cross-inoculation with heterologous baculovirus isolates has been shown relatively consistently to result in the production of a different progeny virus (i.e. the host's own virus) (e.g. Longworth & Cunningham, 1968; McKinley *et al*., 1981; Cory *et al*., 2000). This could be confirmed using restriction enzyme analysis of the DNA of both the virus inocula and progeny virus (Jurcovicova, 1979; Hughes *et al*., 1993). Thus cross-inoculation using virus of two closely related and sympatric host species is an excellent tool for elucidating the presence and extent of latent baculovirus infection, and for ascertaining whether it is likely to be re-activated under realistic field conditions.

In the work reported here, bioassays of field-collected insects were used to determine the cross-infective potential of the two tent caterpillar viruses and whether a re-activatible form of latent virus is present in these field populations. Restriction enzyme analysis was then used to characterise the genomes of *M. c. pluviale* nucleopolyhedrovirus (McplNPV) and *M. disstria* nucleopolyhedrovirus (MadiNPV) to confirm the identity of the progeny virus in the cross-infections and compare the variation in the original inoculum and the progeny viral isolates. The polymerase chain reaction was used to confirm the purity of the inoculum. During the study, populations of western tent caterpillars were low while those of forest tent caterpillars were high, so it was possible to use this procedure to test for the existence of latent or sublethal infection in populations in two different phases of the typical 8–13 years population cycle.

Methods

Insects

The larvae used in the bioassays were hatched from egg masses collected from the field populations during spring

1999. *Malacosoma californicum pluviale* egg masses were collected from red alder trees *Alnus rubra* on Pender and Galiano Islands in the southern Gulf Islands of British Columbia, Canada (48°N, 123°W). Caterpillar populations on these islands reached peak densities in 1996 and declined through 2000. *Malacosoma disstria* has not been reported to occur on these islands but has been seen occasionally in the general vicinity at very low densities in Victoria and on the south-western mainland of British Columbia (J. H. Myers, pers. obs.). *Malacosoma disstria* egg masses were collected from quaking aspen *Populus tremuloides* at various sites around Prince George, British Columbia, ≈600 km north of the western tent caterpillar populations (53°N, 122°W). Populations in this area were at high densities from 1996 to 2000 and declined in 2001 (J. H. Myers, pers. obs.). *Malacosoma californicum pluviale* is rarely observed in the Prince George region but the distributions of the two species do overlap across northern Canada (Myers, 1993).

Egg masses are nearly impossible to find when densities are low. Therefore, in 2000 and 2001 western tent caterpillar larvae were collected from Galiano Island and challenged with forest tent caterpillar virus in the laboratory. No caterpillars could be found on Pender Island in these years.

Virus

McplNPV and MadiNPV were isolated from insects infected naturally in the field in 1998. Virus stocks used for each bioassay were a mixture of occlusion bodies from five infected individuals each collected from different host populations. Occlusion bodies were prepared by macerating individual infected cadavers in 500 µl of dH_2O followed by a series of low speed centrifuge spins (62 ***g*** for 35 s) and washes with dH_2O to separate the occlusion bodies from insect debris. Occlusion bodies were then pelleted at 12 100 ***g*** for 20 min, washed twice with dH_2O, and re-suspended in 1 ml of dH_2O. Equal amounts of occlusion body mixture from each individual were combined to make each virus stock. Stock concentrations were estimated from 10 independent occlusion body counts using a Neubauer Haemocytometer (Hausser Scientific Partnership, Horsham, Pennsylvania).

Bioassays

Each egg mass was surface-sterilised with 0.5% sodium hypochlorite solution (60 s) and rinsed with dH_2O (60 s) to remove virus or bacteria that may have been present as surface contaminants. Egg masses were left to hatch at room temperature. Once larvae began to emerge, they were placed as family groups in 1-l paper cups where they were fed fresh alder leaves and monitored daily. Alder leaves were washed with 0.5% sodium hypochlorite solution and rinsed with dH_2O prior to use. *Malacosoma disstria* larvae were challenged with both McplNPV and MadiNPV in the third, fourth, and fifth instar. The fifth-instar bioassays were replicated three times and bioassays on third and fourth instars once. As fewer *M. c. pluviale* larvae were available, it was only possible to carry out a single bioassay for each virus on third-instar larvae. Prior to starting the bioassays, family groups were mixed and larvae used in the bioassays were removed immediately following the respective moults. Larvae were starved for 24 h then individually fed nucleopolyhedrovirus contaminated alder leaf discs (50 mm^2). Cross-infection doses ranged from 130 to 6600 occlusion bodies per larva for third-instar *M. c. pluviale* and 120–23 000 occlusion bodies per larva, depending on instar, for *M. disstria* and were designed to assess the effect of comparable doses of virus on the two species. Choice of doses was based on the work of Rothman (1995) and Ebling and Kaupp (1997). Control larvae were fed leaf discs treated with dH_2O. Any larvae that did not eat 95% of the leaf disc in 24 h were removed from the study. The number of insects challenged per dose ranged from seven to 24. Because tent caterpillars are naturally gregarious (and separating them can increase mortality), larvae having received the same virus dose were reared in a single group in 0.5-l paper cups with decontaminated alder leaves. Each bioassay was monitored daily for 14 days. In most treatments, insects started dying from virus infection at 6 days post-inoculation, thus only infections that occurred up to day 11 were recorded in the data set because insects dying after this point could have resulted from a secondary wave of infection. The exception to this was fifth-instar forest tent caterpillars inoculated with McplNPV, which did not start dying until day 10 and were monitored until day 17. Nucleopolyhedrovirus infection was diagnosed by physical characteristics (the presence of a fragile cuticle and pale-coloured, milky body contents). The presence of occlusion bodies was verified using light microscopy if diagnosis was uncertain. All dead larvae were stored individually at −20 °C.

Cross-inoculations of western tent caterpillars in 2000 and 2001

Third- and fourth-instar *M. c. pluviale* larvae were collected from the Galiano population in 2000 and 2001. In 2000, the year of lowest density, larvae were collected from five family groups to a total of 158 larvae. Half of these were kept as controls and half were challenged with a single dose of 89 500 occlusion bodies of MadiNPV on a leaf disc. In 2001, third- and fourth-instar larvae were collected from 14 families to a total of 300 larvae; 184 of these were maintained as controls and 126 were challenged with 7850 MadiNPV. Larvae were reared for 2 weeks at room temperature in groups of five in 30-ml plastic cups and fed daily with decontaminated alder leaves.

DNA extraction and restriction endonuclease analysis

Viral DNA was extracted by purifying occlusion bodies from larvae as described above then heating the mixture at

65 °C for 30 min to denature any insect DNases present. Virions were released from occlusion bodies by treatment with an alkali lysis solution (1 M Na_2CO_3, 150 mM NaCl, 0.1 mM EDTA, pH 10.8) at 37 °C for 60 min, after which they were pelleted at 12 100 ***g*** for 30 min. DNA was released from the virions by re-suspending the pellet in 500 µl of proteinase K buffer [10 mM Tris (pH 7.4), 10 mM EDTA, 150 mM NaCl, 0.4% SDS] and 20 µl of proteinase K enzyme (20 mg ml^{-1}) and leaving the mixture overnight at 37 °C. Proteins were removed using a protein precipitation solution (Puregene™, Centra System Inc., Minneapolis, Minnesota). DNA was precipitated in 100% ethanol and sodium acetate (3 M, pH 8.0) for 3 h at 20 °C and pelleted at 12 100 ***g*** for 15 min, followed by a wash with 70% ethanol. The DNA was then re-pelleted and re-suspended in 65 µl of TE buffer. All DNA was stored at 4 °C.

Genetic comparisons between the McplNPV and MadiNPV genomes were made using *Eco*RI, *Hin*dIII, and *Xho*I (GIBCO BRL, Carlsbad, California). Viral DNA (≈2 µg) was digested for 12 h at 37 °C using the conditions recommended by the suppliers and the resulting fragments were separated on 0.7% agarose gels run at 65 V for 24 h. Both the gel and running buffer (1 X TBE) 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, Mississauga, Ontario). Progeny viruses produced in each bioassay (and each individual larva) were characterised using *Hin*dIII to identify the virus and to compare its profile with the isolate used to initiate infection.

Polymerase chain reaction amplification of MadiNPV-specific sequences

In order to confirm the purity of the McplNPV stock, polymerase chain reaction primers were designed to distinguish between MadiNPV and McplNPV by targeting specifically a 443-bp region of the 0.9 kb MadiNPV DNA polymerase gene. Primers were synthesised as 20-mers consisting of DNA complementary to the 5′ (5′-CGCCGACAATGATACATTTA-3′; nt 5′-156-175-3′) and 3′ (5′-TGTTGGCGATTCTCTTGATG-3′; nt 5′-579-598-3′) section of the gene (Nielsen *et al*., 2002). Viral DNA was heated for 15 min at 65 °C prior to its addition to each 25 µl reaction. Approximately 50 ng of DNA was added to a polymerase chain reaction mixture that contained a final concentration of 4 mM $MgCl_2$, 2.5 µl of 10 X PCR buffer, 0.2 mM of each dNTP, 0.5 µM of each primer, together with 1.25 U of Taq polymerase (Gibco BRL). Each reaction consisted of one cycle at 95 °C (1.5 min); three cycles at 95 °C (1.5 min), 49 °C (45 s), and 74 °C (1 min); 32 cycles at 95 °C (1.5 min), 52.5 °C (45 s), 74 °C (1 min); and a final extension at 74 °C for 5 min. These primers detected consistently 0.001 ng of MadiNPV DNA (data not shown). Primers targeting a 1.2 kb region of the McplNPV DNA polymerase gene were used as a positive control for McplNPV. The primers used to amplify this 1.2 kb fragment produced an additional 0.9 kb fragment nested within the DNA polymerase gene (Nielsen *et al*., 2002). Amplification reactions used total viral genomic DNA isolated from one of three sources: MadiNPV- or McplNPV-infected larvae, MadiNPV virus stock, or McplNPV virus stock. Negative controls used all reagents except template DNA.

Statistical analysis

The dose–mortality responses were analysed using Proc Logistic in SAS (1990). Slopes and intercepts were compared as described by Collett (1991). This involved fitting three models; the first fitted model constrained all lines to have the same slopes and intercepts, the second fitted a common slope but allowed the intercepts to vary, the third allowed both the slope and intercept to vary. The difference between the deviance of the third and second model had a chi-square distribution under the null hypothesis that the slopes were identical. The degrees of freedom were given by the number of parameters estimated in the third model minus the second model. All tests of significance were at $\alpha = 0.05$.

Results

Genetic comparison of McplNPV and MadiNPV

Restriction endonuclease profiles of McplNPV genomic DNA were distinct from MadiNPV genomic DNA (Fig. 1). Few common bands were shared between the two virus types. MadiNPV was estimated at 129.4 kb and McplNPV at 122.5 kb.

Bioassays

Malacosoma disstria *larvae infected with MadiNPV*. Only third-instar *M. disstria* larvae showed a significant dose–response to infection with MadiNPV ($F = 41.65$, $P < 0.01$) (Fig. 2) however mortality at all doses for fourth (Fig. 3) and fifth (Fig. 4) instars challenged with MadiNPV was close to or over 50% ($F = 4.69$, $P =$ NS). The LD_{50} for third-instar *M. disstria* larvae challenged with MadiNPV was 1629 (724–4570) occlusion bodies per larva. No mortalities were observed in controls.

Cross-inoculation of M. disstria *larvae with McplNPV*

Malacosoma disstria larvae were apparently susceptible to McplNPV at third (Fig. 2), fourth (Fig. 3), and fifth (Fig. 4) instars but the dose–mortality relationships were not significant for any instar (third instar, $F = 0.55$, $P =$ NS; fourth instar, $F = 3.97$, $P =$ NS; fifth instar, $F = 1.83$, $P =$ NS). The level of mortality produced by McplNPV inoculation was less than that elicited with an

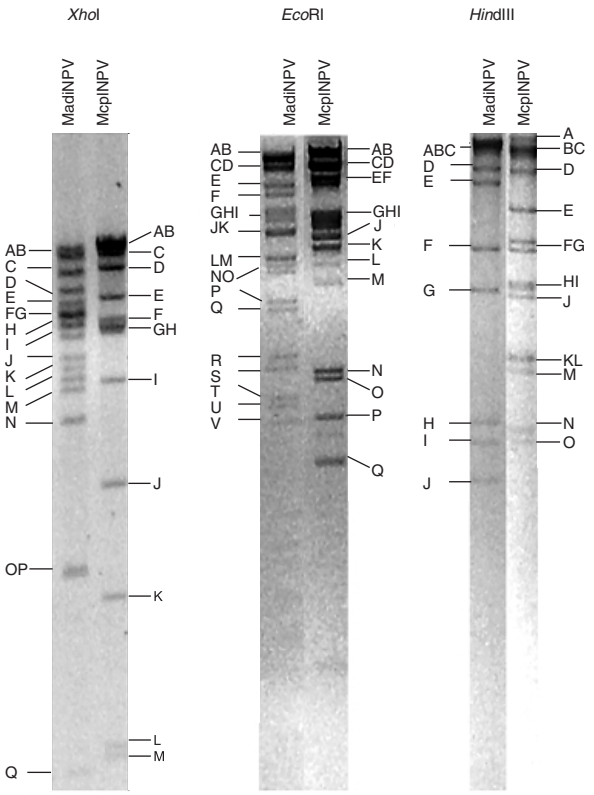

Fig. 1. *Xho*I, *Eco*RI, and *Hin*dIII restriction endonuclease digests of MadiNPV and McplNPV DNA.

equivalent dose of MadiNPV, but ranged from 0 to 50% of the insects challenged. No mortalities were observed in controls. Time to death varied from 6 to 14 days in the bioassays using *M. disstria* larvae and both viruses, however

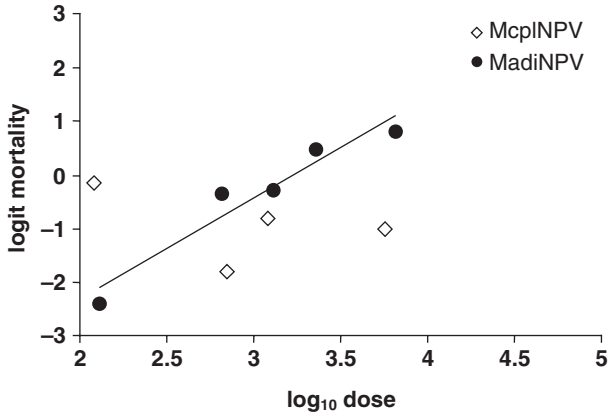

Fig. 2. Mortality of third-instar *Malacosoma disstria* larvae challenged with MadiNPV (●) and McplNPV (◇). Symbols represent actual mortality. $n = 12$, 12, 14, 13, and 13 for MadiNPV for $\log_{10}$ doses of 2.11, 2.82, 3.11, 3.36, and 3.82, and $n = 13$, 11, 12, 12, and 16 for McplNPV for $\log_{10}$ doses of 2.08, 2.84, 3.08, 3.36, and 3.76. MadiNPV challenge: logit (mortality) $= -6.0923 + 1.887$ ($\log_{10}$ dose).

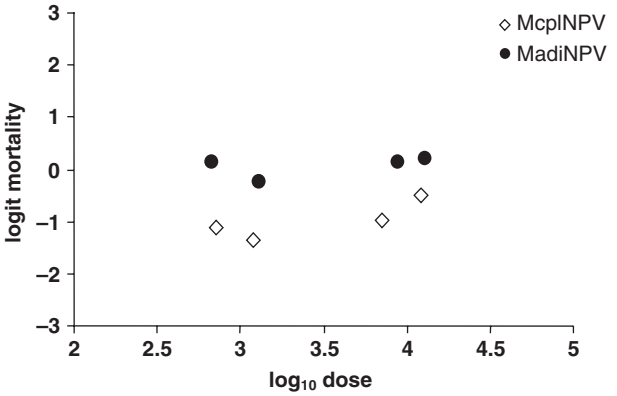

Fig. 3. Mortality of fourth-instar *Malacosoma disstria* larvae challenged with MadiNPV (●) and McplNPV (◇). $n = 15$, 15, 12, and 18 for MadiNPV for $\log_{10}$ doses of 2.85, 3.08, 3.85, and 4.08, and $n = 15$, 15, 12, and 18 for McplNPV for $\log_{10}$ doses of 2.84, 3.11, 3.94, and 4.11.

no consistent pattern was observed with the viruses or instars (data not shown).

Malacosoma californicum pluviale *larvae inoculated with McplNPV*

Mortality did not increase significantly with increasing dose of McplNPV in third-instar *M. c. pluviale* ($F = 1.91$, $P =$ NS) (Fig. 5). No mortalities were observed in controls.

Cross-inoculation of M. c. pluviale *larvae with MadiNPV*

Third-instar *M. c. pluviale* larvae were not susceptible to cross-infection with MadiNPV at the doses given (Fig. 5). A small number of larvae died from unknown causes. In 2000, none of the western tent caterpillars challenged with the MadiNPV died of viral infection although four of the

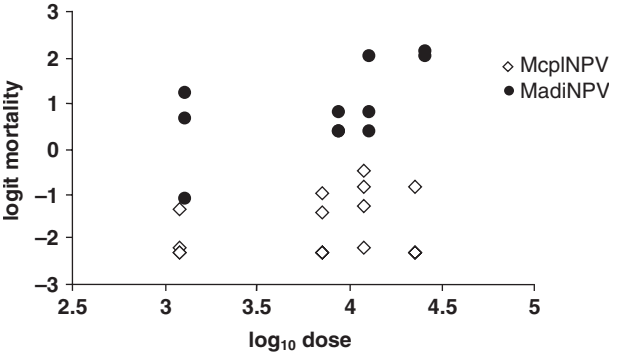

Fig. 4. Mortality of fifth-instar *Malacosoma disstria* challenged with MadiNPV (●) and McplNPV (◇). $n = (8, 9, 9)$ (10, 10, 8) (10, 9, 10), and (9, 10, 10) for MadiNPV for $\log_{10}$ doses of 3.11, 3.94, 4.11, and 4.41, and $n = (10, 9, 10)$ (9, 10, 9) (7, 8, 4), and (9, 9, 7) for McplNPV for $\log_{10}$ doses of 3.08, 3.85, 4.08, and 4.36.

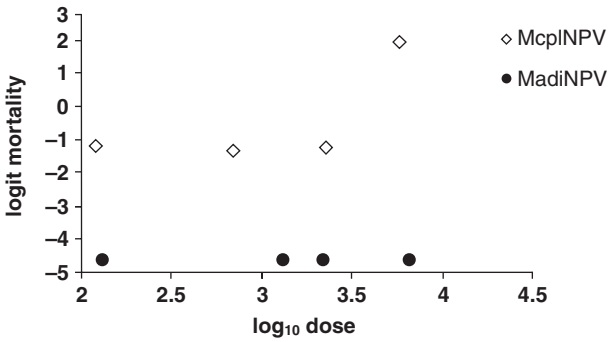

Fig. 5. Mortality of third-instar *Malacosoma californicum pluviale* larvae challenged with MadiNPV (●) and MclpNPV (◇). $n = 21$, 24, 26, and 24 for MadiNPV for $\log_{10}$ doses of 2.11, 3.11, 3.36, and 3.82, and $n = 20$, 20, 21, and 20 for MclpNPV for $\log_{10}$ doses of 2.08, 2.84, 3.36, and 3.76. No viral mortalities were observed from the cross-infection of *M. c. pluviale* with MadiNPV.

control larvae died of nucleopolyhedrovirus disease. Controls in this year were from field-collected larvae. In 2001, none of the cross-inoculated or control larvae died of virus.

Bioassays: examination of progeny virus

Restriction endonuclease analysis was used to verify that the mortalities in the cross-infections were caused by the virus inoculum used to initiate infection. Overall, 59 of the *M. disstria* larvae challenged with MclpNPV died of nucleopolyhedrovirus infection. Restriction endonuclease profiles

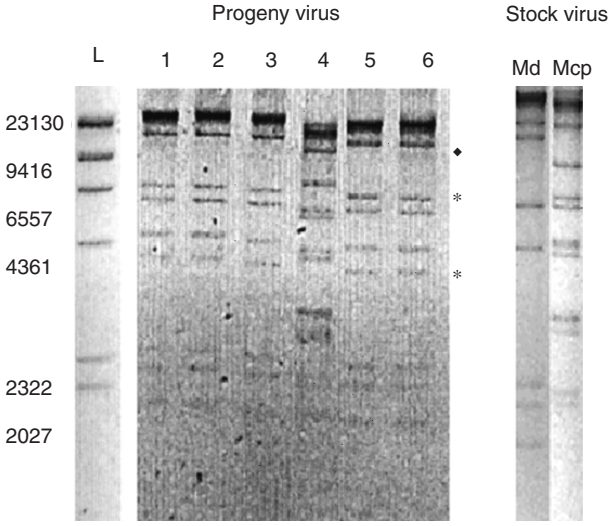

Fig. 6. *Hin*dIII restriction endonuclease profiles of virus DNA extracted from individual *Malacosoma disstria* larvae challenged with MclpNPV (lanes 1–6). L is lambda DNA size marker cut with *Hin*dIII. Also shown are *Hin*dIII profiles of stock virus DNA for both MadiNPV (Md) and MclpNPV (Mcp). Additional bands in the progeny virus, as compared with the MadiNPV stock, indicated by * and missing bands ◆.

of virus DNA successfully isolated from 50 of these larvae demonstrated that only six individuals actually died from MclpNPV infection. DNA extracted from the remaining nine larvae could not be cut with restriction endonucleases and thus it was not possible to ascertain which virus had killed them. Two variants of MclpNPV were isolated from the six *M. disstria* larvae succumbing to MclpNPV infection (Cooper, 2001). Deaths from MclpNPV infection occurred mainly in larvae that had been treated in the fifth instar (five out of six insects) and were not related to any specific dose. The remaining 44 larvae that died of virus infection were infected with MadiNPV. The DNA profiles from all 44 of these cadavers were identical (Fig. 6). This virus, while clearly MadiNPV, differed from the MadiNPV inoculum used to challenge the larvae and contained at least three differences in the *Hin*dIII restriction profile (Fig. 6). For comparison, an arbitrary sample of 23 *M. disstria* larvae challenged with MadiNPV was also examined using restriction endonuclease analysis. All individuals examined died from a virus variant identical to the MadiNPV identified in the cross-infection bioassays (data not shown). In previous bioassays with *M. disstria* using mixed genotype MadiNPV stocks, individual larvae were shown to be infected with different virus variants (Cooper, 2001). In contrast, the virus variants produced in *M. disstria* following inoculation with either the MclpNPV or MadiNPV in the current study were identical.

Larvae of *M. c. pluviale* that died of infection following inoculation with MclpNPV were also examined using restriction endonuclease analysis of virus DNA. MclpNPV was confirmed in the 30 larvae examined. DNA profiles of MclpNPV produced during the cross-infections were genetically variable but similar to the MclpNPV stock inoculum used (Fig. 6) (Cooper, 2001).

PCR examination of MclpNPV stock

To confirm that the initial MclpNPV stock was not contaminated with MadiNPV, the virus stock was tested using the polymerase chain reaction. No MadiNPV was detected in the MclpNPV stock (Fig. 7). MclpNPV was also confirmed in the MclpNPV samples in lanes 6–10 using the MclpNPV DNA polymerase primers (data not shown).

Discussion

The current study shows that the nucleopolyhedroviruses of forest and western tent caterpillars are genetically distinct, that the nucleopolyhedrovirus from western tent caterpillars can infect forest tent caterpillars, and, most importantly, that cross-inoculation with MclpNPV can activate a latent or persistent infection in forest tent caterpillars from high-density populations. The reciprocal cross-inoculations, i.e. western tent caterpillars with forest tent caterpillar virus, resulted in neither overt nor activated viral infection in larvae collected in 3 years of low population

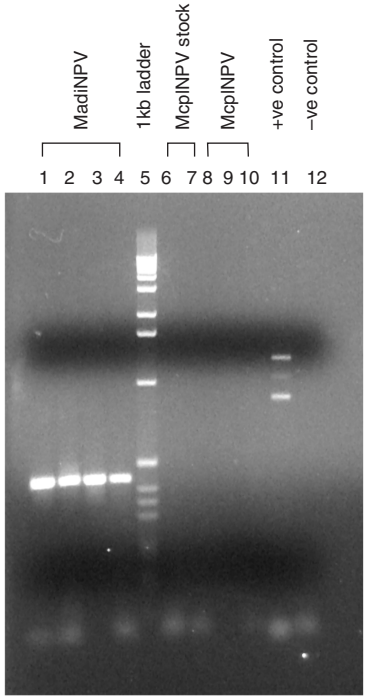

Fig. 7. Agarose gel showing the results of the examination of the McplNPV stock for MadiNPV contamination using the polymerase chain reaction. Lanes 1–4, MadiNPV DNA isolated from field-collected *Malacosoma disstria* larvae; lanes 6 and 7, McplNPV stock inoculum DNA; lanes 8, 9, and 10, McplNPV DNA from infected *Malacosoma californicum pluviale*; lane 11, purified McplNPV DNA; lane 12, negative control dH_2O. All samples were probed with MadiNPV DNA polymerase specific primers, except lane 11, which was probed with McplNPV DNA polymerase primers.

density. This is one of the first controlled experiments carried out on field populations to demonstrate the activation of latent infection, and the first to try to relate the results to changes in host and pathogen population density.

The restriction endonuclease profiles of McplNPV and MadiNPV have not been compared before nor has cross-infection of western tent caterpillars with MadiNPV been attempted. There is, however, earlier work on *M. disstria* and its nucleopolyhedrovirus. Two groups have characterised various isolates of MadiNPV; Keddie and Erlandson (1995) gave an estimated genome size of 130.4 kb, and Ebling and Kaupp (1995) estimated the size to be 85 kb. In neither case were the *Hin*dIII restriction fragment patterns identical to those shown here, and that of Keddie and Erlandson (1995) has considerably fewer bands than the isolate shown here, which has an estimated size of 129.4 kb. There are few comparable bioassay data for either species; Ebling and Kaupp (1997) cited an LD_{50} value of 20 000 occlusion bodies for fourth-instar *M. disstria* infected with MadiNPV, and Frid and Myers (2002) measured the LD_{50} of third-instar *M. c. pluviale* at ≈1000 occlusion bodies. In the current study, an LD_{50} of 1629 occlusion bodies was estimated for third-instar *M. disstria* larvae infected with MadiNPV and mortality in fourth- and fifth-instar *M. disstria* was consistently above 50% in the bioassays, even with doses as low as ≈1000 occlusion bodies. This suggests that MadiNPV may also have triggered latent virus even at low density.

Stairs (1964) reported equal susceptibility of first- and second-instar forest tent caterpillars to MadiNPV and McplNPV, however techniques were not available to confirm the identity of the progeny virus and the current data indicate how important molecular techniques are in identifying true cross-infection.

There are three possible explanations for the production of MadiNPV in insects challenged with McplNPV: low levels of MadiNPV contamination of the McplNPV inoculum, low levels of contamination occurring during laboratory rearing, or a latent infection being activated in *M. disstria* larvae. Several lines of evidence suggest the last. (1) Inoculating *M. disstria* larvae with both MadiNPV and McplNPV produced virus with the same MadiNPV restriction endonuclease profile and this differed from the genotype of the MadiNPV used as the inoculum. The MadiNPV stock consisted of a collection of occlusion bodies from five different field-infected individuals. DNA analysis of virus isolated from field-infected *M. disstria* larvae has shown that MadiNPV is genetically heterogeneous both within and between collection sites (Cooper, 2001). Previous experience with mixed genotype inocula of both MadiNPV and McplNPV has shown that not all individuals are necessarily killed by the same genotype (Cooper, 2001). Therefore all mortalities would not be expected to be the result of the same genetic variant of MadiNPV if they resulted from contamination. (2) Polymerase chain reaction examination of the McplNPV stock failed to find evidence that MadiNPV DNA was present. (3) External contamination is extremely unlikely because the egg masses were decontaminated before larvae began to hatch. Incomplete decontamination, laboratory contamination, and contamination of foliage are also unlikely because no mortalities were seen in controls.

The traditional course for baculovirus infection is thought to be via horizontal transmission mediated by occlusion bodies surviving in the environment. Given that many insect species do not reach outbreak densities, however, the vertical transmission of baculovirus infections may be crucial for long-term virus persistence (Kukan, 1999; Burden *et al.*, 2002). Vertical transmission not only provides a mechanism for the transfer of virus from one generation to the next, it also provides a mechanism for the movement of virus to new areas via host dispersal. For example, nucleopolyhedrovirus is associated with populations of western tent caterpillars that invade new sites at the time of regional peak densities (Myers, 2000; Cooper, 2001). There is considerable evidence that baculoviruses can be transmitted vertically from adult to offspring (Kukan, 1999), however this process appears to vary among viruses and species. Vertical transmission can include the passage of overt disease, either a result of virus contamination on the surface of the egg from the adult (or sometimes the environment) or by an active virus infection being passed within

the egg (transovarially). These two processes can be differentiated by surface-sterilising the outside of the eggs prior to egg hatch (Neeglund & Mathad, 1978; Abul-Nasr *et al*., 1979; McKinley *et al*., 1981; Young & Yearian, 1982; Smits & Vlak, 1988; Murray & Elkinton, 1990; Fuxa & Richter, 1991; Kukan, 1999; Myers *et al*., 2000). Infected larvae originating from surface-sterilised egg masses are likely to be the result of transovarially transmitted virus.

Vertical transmission helps to explain low levels of infection in field-collected egg masses and provides an explanation for the inability to consistently rear healthy laboratory stocks in other species, such as the cabbage looper *Trichoplusia ni* (McEwen & Hervey, 1960). More recently, Fuxa *et al*. (1999) demonstrated transovarial transmission of both nucleopolyhedrovirus and cypovirus in laboratory colonies of *T. ni* larvae after decontamination of egg mass surfaces. Rothman (1995) observed a low level of nucleopolyhedrovirus infection in 24% of 25 western tent caterpillar families in which the parents had been reared at high density and exposed to nucleopolyhedrovirus in field experiments, and the offspring reared in the laboratory from surface-sterilised egg masses. Kukan (1999) reviewed four studies, including two of western tent caterpillars, that examined progeny from field-collected egg masses and, in each case, surface sterilisation of the eggs was found to reduce but not eliminate the incidence of virus disease (Doane, 1969; Bell *et al*., 1981; Kukan, 1996; L. Rothman and J. H. Myers, unpublished). Nucleopolyhedrovirus infection was found in 9% of surface-sterilised egg masses collected from a high-density western tent caterpillar field population and in 2–2.5% of larvae reared from decontaminated egg masses. This compares with 22% of unsterilised, control egg masses producing infected larvae. These experiments suggest that low levels of overt nucleopolyhedrovirus infection may be transmitted vertically within the eggs themselves in tent caterpillar populations in the field, but the viruses were not characterised.

It has frequently been suggested that sporadic baculovirus outbreaks in natural lepidopteran populations (and laboratory cultures) result from the activation of latent infections (Longworth & Cunningham, 1968; Podgwaite & Mazzone, 1986; Cory *et al*., 1997). Latency is used to describe the state of a virus that does not produce obvious signs of infection, including sublethal effects, but can be transmitted between host generations (Hale & Margham, 1988). This requires the pathogen to remain non-replicating and non-infective until an appropriate stress or stimulus can activate the infective form. Several mechanisms for latent viruses have been suggested. For example, *Herpes simplex* virus infecting humans is maintained within the host nucleus as independent viral genetic material (Mellerick & Fraser, 1987), while Hepatitis B virus can be integrated directly into the host genome (Howard, 1986). In contrast, the measles virus can be maintained as a low-level persistent infection (Catteneo *et al*., 1988).

Information on whether baculoviruses can persist in a truly latent form is limited because the location and the nature of the virus were not determined in early studies, in part because suitable *in vitro* systems and techniques were not available to discriminate between baculovirus isolates with certainty. The occluded nature of the viruses also made it difficult to rule out external contamination in many circumstances. Now there is a wide range of molecular techniques that can be used to investigate the presence of sublethal levels of virus infection. Earlier studies of high-density forest tent caterpillars used DNA probes against the polyhedrin gene to look for latent virus, and found evidence for nucleopolyhedrovirus DNA in 0.5% of field-collected forest tent caterpillar pupae but not in adults (Kukan, 1996, 1999). A more sensitive polymerase chain reaction assay based on the detection of a random (non-polyhedrin) DNA fragment detected nucleopolyhedrovirus in 6% of pupae, but in no adult western tent caterpillars arising from larvae collected from populations that were beginning to increase in density (Kukan, 1999). Therefore molecular evidence suggests that nucleopolyhedrovirus can be maintained at low levels through the pupal stage of both tent caterpillar species. It is possible that virus is lost at the adult stage or that more sensitive extraction techniques and detection assays may be necessary for this stage. Probing larvae is not a reliable measure of detecting sublethal infection as it is not known what proportion will go on to develop overt nucleopolyhedrovirus infection, and such studies need to be combined with rearing experiments. Eastwell *et al*. (1999), using the polymerase chain reaction, observed that 23% of wild codling moth *Cydia pomonella* larvae in the apple-growing region of British Columbia carry a granulovirus even though levels of overt infection were low. This virus also persists in laboratory cultures.

Few studies have actually investigated the nature of latent infections in insect cultures and none has studied field populations. Hughes *et al*. (1993, 1997) investigated a latent nucleopolyhedrovirus infection reported to be present in a long-term laboratory culture of the cabbage moth *Mamestra brassicae*. They demonstrated that the culture contained a low level persistent infection of *M. brassicae* nucleopolyhedrovirus (MbMNPV) that could be activated when insects were fed the closely related *Panolis flammea* nucleopolyhedrovirus and the more distantly related *Autographa californica* nucleopolyhedrovirus. Transcripts for early (*ie*-1), late (p6.9), and very late (polyhedrin) gene expression were found in fat body cells of larvae, which implied that infection was maintained in a persistent state, allowing the continuous expression of viral proteins at low levels. This is analogous to the situation proposed to explain persistent measles infections (Catteneo *et al*., 1988).

Very recently, a similar low level persistent infection was detected in the Indian meal moth *Plodia interpunctella* (Burden *et al*., 2002) but in this case the infection was introduced by inoculating late-instar larvae with a baculovirus. Ribonucleic acid transcripts for the granulin protein of the *P. interpunctella* granulovirus were present in a high proportion of *P. interpunctella* larvae, pupae, and adults that survived virus challenge. Granulin is a late-expressed gene that is only transcribed after viral genome replication. In addition, RNA transcripts were detected in 60–80% of second-generation larvae derived from the surviving adults, demonstrating that

sublethal infections can be transmitted vertically to offspring (Burden *et al*., 2002). These data imply that baculoviruses may not exist in a truly latent state but survive between generations as active low-level persistent infections.

Experiments designed to detect and promote persistent infection, to pinpoint sites of persistence, and to identify potential environmental triggers for the re-activation of disease development will provide important clues about the factors that mediate the shift from a virus that kills its host to one that can initiate a sublethal infection and vice versa (Myers & Rothman, 1995). Infection by the host's natural virus was triggered by cross-infection in a high proportion of individuals tested from a high-density forest tent caterpillar population. It is also interesting to note that the same virus variant was found in all individuals that died of virus infection (whether a result of homologous or cross-infection) and thus may represent a low virulence genotype adapted for vertical, sublethal transmission; however viral infection was not elicited in larvae from populations of western tent caterpillars late in the decline phase when levels of virus infection were considerably lower. This does not necessarily mean that nucleopolyhedrovirus is not present within these populations; it may be present in a different form that cannot be activated by a second virus.

Reduced fecundity is a characteristic of surviving baculovirus challenge and of declining tent caterpillar populations. Whether the persistent virus found in this study is linked with the observed sublethal effects remains to be determined (Rothman & Myers, 1994). The increasing number of examples of persistent, sublethal baculovirus infections in Lepidoptera (J. S. Cory, pers obs.) may indicate that long-term virus maintenance does not incur a cost to the host and that major sublethal effects, such as significant reductions in fecundity (Rothman & Myers, 1996), may primarily be a more immediate consequence of surviving a baculovirus challenge. These data have broad implications for understanding the role that baculoviruses may play in insect population dynamics and the potential relevance of sublethal infections in other field populations.

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