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REPORT

Adaptation in an insect host–plant pathogen interaction

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Abstract

Selection on parasites to adapt to local host populations may be direct or through other components of the system such as vectors or the food plant on which the parasite is ingested. To test for local adaptation of nucleopolyhedrovirus among island populations of western tent caterpillars, *Malacosoma californicum pluviale*, we compared virus isolates from three geographically distinct sites with different dominant host plants. Pathogenicity, speed of kill and virus production of each isolate were examined on the three food plants. Virus isolates from the two permanent host populations had the fastest speed of kill on the host plant from which they were isolated. This was not the case for a caterpillar population that goes extinct when populations are regionally low. Virus isolates on some plant species combined rapid speed of kill with high virus yield. Infection of hosts by mixed microparasite populations could facilitate local adaptation in response to differing food plant chemistry.

Keywords

Baculovirus, fitness, genotype × environment, local adaptation, microparasite, nucleopolyhedroviruses, tent caterpillars, trade-offs, tritrophic interaction, virulence.

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INTRODUCTION

Geographical heterogeneity in host–parasite interactions is thought to play a major role in co-evolutionary processes (Thompson 1997, 1999; Dybdahl & Storfer 2003) and the maintenance of variation in host and parasite populations (Nuismer *et al.* 1999). Parasites are usually more numerous, have shorter generation times and are likely to mutate more often than their hosts. Thus they are predicted to be able to adapt to local host populations (Ebert 1994). Local adaptation of parasites to hosts has been demonstrated (e.g. Lively & Dybdahl 2000; Thrall *et al.* 2002), but is not always found (e.g. Koskela *et al.* 2000; Kaltz & Shykoff 2002; Zhan *et al.* 2002). However, parasites could also be influenced by genotype × environment interactions in that ecological conditions can influence pathogens directly, and possibly in some circumstances to a greater extent than the host. This is particularly likely to be the case with parasites that persist outside their host and are primarily transmitted horizontally via environmental contamination of foliage, such as occurs for many insect microparasites, e.g. bacteria, fungi, viruses, protozoans.

For insects that are polyphagous, host plant species can potentially influence the infection by microparasites in a variety of ways. Plant architecture can alter environmental persistence, plant palatability can modulate host feeding behaviour and thus the probability of ingesting infectious units, and plant chemistry can influence infection processes and post-digestive resistance mechanisms (review in Cory & Myers 2003). For pathogens that must be ingested to initiate infection, attention has been on plant chemistry influences that occur in the mid-gut where infection is initiated (Maksymiuk 1970; Keating *et al.* 1988; Duffey *et al.* 1995; Carloye *et al.* 1998; Hoover *et al.* 1998a,b). In nucleopolyhedroviruses (NPVs) (Baculoviridae) in particular, an increasing number of studies have shown that host plant can influence a range of key fitness traits including host mortality, time to death (speed of kill of the pathogen) and the production of infectious stages (Farrar & Ridgway 2000; Ali *et al.* 1998, 2002; Raymond *et al.* 2002). Additionally, entomopathogenic nematode infection has been shown to be influenced by plant chemistry (Barbercheck 1993; Barbercheck *et al.* 1995) and even to show adaptation to hosts that have been fed on different food plants (Barbercheck *et al.* 2003).

Genetic diversity and high levels of mutation or recombination will also influence the capacity of a pathogen to adapt to plants. Many entomopathogens show high levels

of genotypic and phenotypic diversity at a variety of scales from the individual to population level and larger geographical areas (e.g. Gettig & McCarthy 1982; Laitinen *et al.* 1996; Berretta *et al.* 1998; Helgason *et al.* 1998; Blouin *et al.* 1999; Hodgson *et al.* 2001; Cooper *et al.* 2003). However, while the effect of different crop plants is sometimes considered in the application of microbial insecticides, the influence of host food plant on the distribution or fitness of pathogen isolates or individual variants has received little attention. The exception to this is the recent demonstration that host plant species can exert a differential selective effect on individual NPV genotypes (Hodgson *et al.* 2002). We thus predict that an entomopathogen, such as a baculovirus, could adapt to locally abundant host plants and the plant species on which the insect host feeds could be an important selective force in insect–pathogen relationships.

To address the issue of adaptation to locally abundant host food plants we used insect baculoviruses, a group for which host plant effects are known to be important (Cory & Myers 2003). NPVs, primarily pathogens of larval Lepidoptera, usually kill their hosts and the infectious transmission stages are not released until the host dies. These transmission stages (or occlusion bodies, OBs) are composed of multiple DNA genomes occluded within a protein coat. This structure enables NPVs to survive outside of their host for long periods, particularly when they are protected from ultraviolet irradiation. Susceptible caterpillars acquire infection by ingesting OBs from contaminated surfaces, usually host plants, thus the major route of virus transmission is horizontal [although baculoviruses can be transmitted vertically (Kukan 1999; Cory & Myers 2003)]. Thus in a stable environment, such as that provided by perennial plants, baculoviruses can potentially persist from one year and one host generation to the next on the plant surface. In this way the baculovirus is intimately linked with the food plants of its hosts.

Baculoviruses have been associated with the population fluctuations of several species of forest Lepidoptera, primarily from the Lymantriidae and the Lasiocampidae (Cory & Myers 2003), and have been implicated as a potential influence on cyclic population dynamics (Myers 1988). One of the best studied field systems is the gregarious western tent caterpillar, *Malacosoma californicum pluviale* (Dyar). In western Canada, *M. c. pluviale* undergoes population cycles every 7–10 years and an NPV commonly found in tent caterpillar populations is associated with population declines (Myers 2000). Recent work indicates that *M. c. pluviale* NPV (*Mcp*NPV) populations are genetically diverse, and additionally, that they show hierarchical spatial population structure (Cooper *et al.* 2003). Western tent caterpillars are polyphagous, and in southwestern British Columbia geographically distinct populations (often occurring on islands) feed on different predominant host trees. This therefore provided the scenario to test whether virus from these isolated populations had a higher fitness on the common host plant of the insect population than on alternative host plants.

We collected isolates of NPV from three distinct island populations of the western tent caterpillar in the southern Gulf Islands in Haro Strait; British Columbia, Canada, that differ in their dominant host plant. We collected the isolates at a time when the population density had been declining for several years and the likelihood of host migration from other sites was low. We then asked whether any of three key infection traits thought to influence virus fitness, pathogenicity, time to death or production of infectious units (OBs) was differentially affected by the host plant on which the virus dose was administered. Specifically, we asked, did the virus isolates perform 'better' on their host plant of isolation?

Although the Saturna and Mandarte sites supported continuous, fluctuating populations of tent caterpillars (and NPV), tent caterpillar populations only occur at the Montague site following regionally high insect densities (and thus this is probably a sink site in which populations occasionally originate from surrounding areas where larvae predominately feed on red alder). We thus predicted that whereas the NPV populations from Saturna and Mandarte have had the potential for adaptation to the locally abundant host plant species, the virus from Montague is not likely to demonstrate adaptation to the local food plant species, but is likely to have intermediate levels of adaptation following importation from other sites. We show that for one of the infection traits, speed of kill (time to death) virus isolates perform best on the host plant on which they were isolated. This relationship did not hold however for the virus collected from the sink population. Additionally, we demonstrate for the most effective combinations, that increased speed of kill was achieved without the expected cost in terms of reduced levels of virus production.

MATERIALS AND METHODS

Insects

Six western tent caterpillar egg masses with *c.* 200 eggs each were collected from Point Roberts, USA in March 2001 and were stored at 4 °C until 2 May 2001. This is not one of the long-term study populations and has not been exposed to any of the virus isolates used in the study, thus avoiding the possibility that the insects could be adapted to the virus isolates used. The predominant food plant at this site is red alder. Egg masses were surface sterilized to remove any pathogens that might be contaminating the outside of the eggs by soaking for 5 min in a 0.5% hypochlorite solution followed by copious rinsing in tap water. Larvae were reared

as family groups at room temperature until the third instar on fresh alder leaves decontaminated by rinsing in dilute hypochlorite solution. There were no virus deaths in any family prior to virus inoculation.

Virus isolates

Virus was obtained from infected caterpillars collected from three populations in 1998 at a point when *M. c. pluviale* populations were in decline and NPV prevalence was low. Each site was on a separate island and each area was dominated by a specific host plant. The three sites were Saturna Island where the larvae feed on red alder (*Alnus rubra* Bong), Mandarte Island where the insects feed predominantly on wild rose (*Rosa nutkana* Presl.) (no alder is present on the island) and Galiano Island (Montague Provincial Park) where the larvae feed on apple (*Pyrus malus* L.) (for further details see Myers 2000; Cooper *et al.* 2003). Western tent caterpillar populations persist even through low density on both Saturna and Mandarte islands, whereas at Montague, Galiano Island, insects are only present when population densities are high on other parts of the island and adjacent islands. This site is apparently colonized by migration during years of peak moth density. Virus was extracted by macerating well-infected, single larvae with distilled H_2O. OBs were separated from insect debris with a series of three washes and low speed centrifuge spins (63 g for 30 s). OBs were then pelleted with a high speed centrifuge spin (12 100 g for 20 min) and re-suspended in 1 mL of distilled H_2O. The virus stocks used as inocula were made by combining 300 μl of OBs from four infected caterpillars collected from separate families in each of the host sites. Stock concentrations were estimated from 10 counts using a Neubauer haemocytometer.

Bioassay experiment

On 18 May 2001 newly moulted third instar larvae were sorted into 54 groups of 20 individuals with equal numbers from the six families in order to homogenize any variation in susceptibility between families. The following day the larvae were placed individually in 1 oz. plastic cups with a leaf disc (50 mm^2) of one of three host plant types, alder, wild rose or apple. Twenty larvae were allotted per virus–plant–dose combination. After 24 h the larvae were challenged with *Mcpl*NPV offered on a disc of the same plant. Five broadly similar virus doses and a water only control were set up, dose range being based on the virus concentration and previous studies with these virus isolates. Each virus dose was recounted after dilution to ensure accuracy. The NPV dose per larva was as follows: Saturna, 2046, 568, 294, 192 and 44 OBs; Mandarte, 5422, 1082, 596, 276 and 36 OBs; Montague, 5546, 488, 234, 166 and 80 OBs. Virus was applied to the leaf disc in 2 μl of distilled H_2O. Larvae that had consumed the leaf disc within 24 h were transferred to fresh 1 oz. plastic cups and fed another leaf disc of the same host plant type. After a further 24 h all larvae were fed ad libitum with alder leaves that had been decontaminated in dilute hypochlorite solution. Larvae were reared individually at room temperature (*c.* 22 °C) and monitored daily. Individuals that died of virus infection were placed in Eppendorf tubes, weighed and frozen. In the few instances where cause of death was unclear a sub-sample of larval macerate was stained with Giesma and examined using light microscopy. The production of OBs was estimated by macerating individual larvae in 1 mL of distilled H_2O and counting two sub-samples using a Neubauer haemocytometer. Larvae that disintegrated after death and could not be transferred to tubes were not used for yield counts.

Statistical analysis

Virus induced mortality was analysed using a binary error structure, using the scale parameter to adjust deviances if required, with generalized linear models (GLIM version 3.77, Royal Statistical Society). Data for time to death and virus yield were transformed with natural logs and $\log_{10}$, respectively before analysis with $\log_{10}$ virus dose being included as a covariate for both analyses and ln speed of kill as an additional covariate for the yield analysis (JMP, SAS Institute). Initially all explanatory variables and their interactions were fitted to the data, the contribution of each term was tested for significance and non-significant terms were removed (Crawley 1993).

RESULTS

Pathogenicity

Mortality increased with increasing virus dose (dose: $F_{2,41} = 74.68$, $P < 0.001$) but there was no difference in pathogenicity between the virus isolates (virus: $F_{2,38} = 3.1$, $P > 0.05$) or on the different host plants (plant: $F_{2,38} = 2.65$, $P > 0.05$). The overall LD_{50} was 1652 OBs per larva (95% confidence intervals, 1148 and 2673 OBs).

Speed of kill

Host plant exerted a significant effect on the time the virus took to kill its host and this was influenced by the provenance of the virus (virus × plant: $F_{2,187} = 6.28$, $P = 0.0023$). Virus dose also had a major influence on time to death which decreased with increasing dose (dose: $F_{1,187} = 6.72$, $P = 0.01$) (Fig. 1). For the NPV isolates from Saturna and Mandarte, the time to death was shortest when the insects were infected with the virus isolate on the host plant

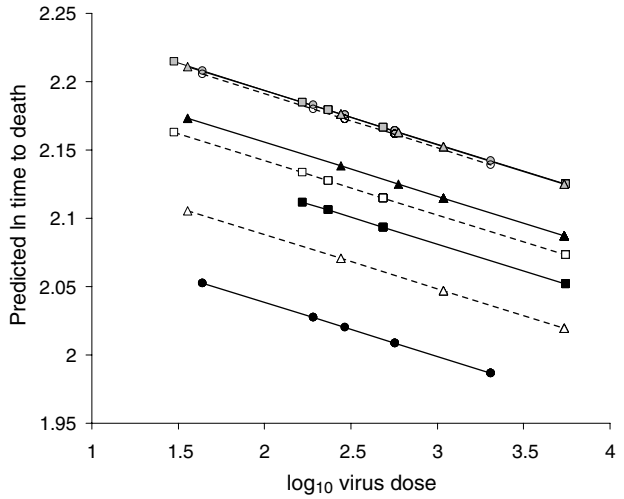

Figure 1 The relationship between time to death and virus dose for third instar *M. c. pluviale* larvae infected with three NPV isolates on three different host plants. Circles represent insects infected with the Saturna isolate, triangles the Mandarte isolate and squares the Montague isolate: closed symbols represent alder, open symbols rose and gray symbols apple. The solid line represents alder and the short dashed line rose. All the apple treatments have been combined. For clarity, only the model lines are shown.

most common on the same site. Thus speed of kill for the Saturna virus was fastest on alder and for the Mandarte virus speed of kill was fastest when the insects were inoculated on rose. The virus collected from Montague on apple did not follow this trend. Time to death for insects inoculated on apple was the same across all virus isolates (grouping this treatment resulted in a non-significant change to the model, $F_{2,185} = 1.224$, $P > 0.05$). Slopes of the dose–time responses between host plants (plant × dose: $F_{2,181} = 1.506$, $P = 0.227$) or virus isolates (virus × dose: $F_{2,181} = 1.131$, $P = 0.325$) did not differ.

Production of occlusion bodies

Yield of virus OBs at death was predicted by a complex relationship involving time to death, virus isolate, plant species and virus dose. Insects that take longer to die tend to be larger and thus produce more virus OBs. Thus the relationship between virus yield, virus isolate and host plant was expected to mirror that found with time to death. However, although time to death was a strong predictor of virus yield within each treatment combination ($F_{1,123} = 21.46$, $P < 0.0001$, yield increased with time to death) this was not the case. Both host plant (plant: $F_{2,123} = 4.864$, $P = 0.009$) and virus isolate (virus: $F_{2,123} = 5.928$, $P = 0.003$) had a significant effect on virus yield, however there was no interaction between the two (virus × plant: $F_{4,119} = 0.6843$, $P > 0.05$). Larvae inoculated on alder produced significantly more virus than those infected on rose and apple ($F_{1,123} = 9.5$, $P = 0.002$). Thus, contrary to what might have been predicted from a general relationship between speed of kill and virus yield, in the case of the Saturna virus–alder combination, the insects that died most rapidly following inoculation on alder also produced a larger quantity of virus than insects that died more slowly after being infected on rose or apple. A similar outcome is seen in the relationship between time to death and yield in insects infected with the Montague isolate on alder. Variation in virus yield was not simply determined by larval development rate associated with the different food plants at the time of inoculation since control larvae for all food plant treatments pupated on average 20 days following treatment. The prediction of virus yield was further complicated by the fact that the virus isolates interacted differently with dose (virus × log dose: $F_{2,123} = 6.42$, $P = 0.002$). The relationship between log yield and log dose increased in a positive linear manner for the Saturna and Montague virus isolates but was negative for the Mandarte isolate (Fig. 2).

Analysis of yield of virus per unit weight of cadaver gives some indication as to whether the different virus isolates have different rates of conversion of insect tissue to virus OBs. No significant differences were found between the

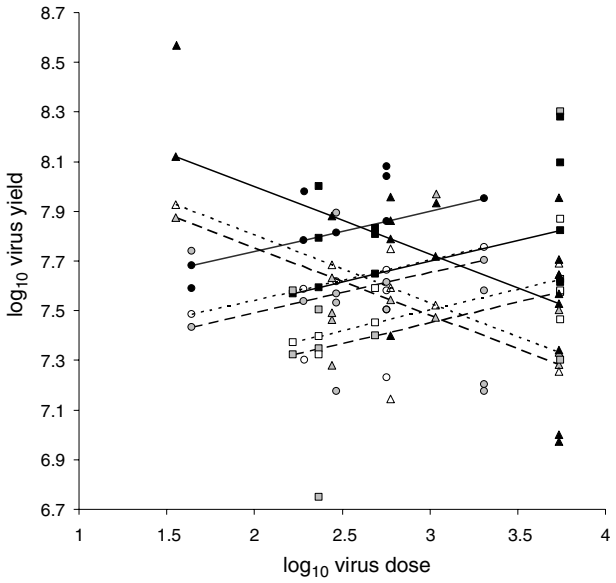

Figure 2 The relationship between yield of virus OBs per cadaver and $\log_{10}$ virus dose for third instar *M. c. pluviale* larvae infected with three NPV isolates on three different host plants. Circles represent insects infected with the Saturna isolate, triangles the Mandarte isolate and squares the Montague isolate: closed symbols represent alder, open symbols rose and gray symbols apple. The solid line represents alder, the short dashed line rose and the longer dashes apple. For clarity the relationship is shown only for a speed of kill of 8 days: virus yield increases with increasing time to death.

treatments (virus: $F_{2,125} = 1$; plant: $F_{2,125} = 0.147$; time $F_{1,125} = 0.063$; dose: $F_{1,125} = 0.863$, all $P > 0.05$). Weight of virus-infected cadavers followed the same trend as virus yield. Larval weight was predicted by host plant, time to death, virus isolate and dose (plant: $F_{2,123} = 7.15$, $P = 0.0012$; virus × dose: $F_{2,123} = 3.34$, $P = 0.039$; time: $F_{1,123} = 49.77$, $P < 0.0001$). This indicates that the differences in yield were primarily a consequence of differences in cadaver weight.

DISCUSSION

The widespread genetic and phenotypic diversity in entomopathogen populations, combined with the strong influence of phytochemicals on the insect–pathogen interaction, provides the opportunity for plant chemistry to have a profound effect on pathogen population structure and evolution. In this study, we have shown that it is possible for a baculovirus population to become adapted to the locally abundant food plant of its host: NPV isolates collected from persistent host populations killed larvae more rapidly when administered on their sympatric plant species. Infectious OBs are not released from the fatally infected insect until it dies. Thus a faster speed of kill means that virus is released from the host more rapidly, and this creates the potential for earlier secondary transmission of disease and more cycles of virus amplification within the single generation of the host. This has clear implications for increased virus fitness (Dushoff & Dwyer 2001). As predicted, the phenotype of the isolate collected from the non-persistent *M. c. pluviale* population at Montague on Galiano Island, did not follow this trend. This virus isolate was most efficacious on alder rather than the local host plant, apple, supporting the proposal that this population was founded by insects (and virus) imported from other populations on the island that predominantly feed on alder.

We had anticipated that the relationship between host plant, virus isolate and virus yield would be predictable from that for speed of kill, with yield being lowest in the larvae that died most rapidly. Our earlier studies have shown that a trade-off usually occurs between the time required for insect death and the resulting yield of OBs such that earlier death results in fewer infectious OBs being produced (Hernández-Crespo *et al.* 2001; Hodgson *et al.* 2001). However, although virus yield increased with time to death within each treatment combination, this was not the case in comparisons across the treatment combinations. Unexpectedly, we found that rapid speed of kill was not necessarily accompanied by reduced virus productivity, with some isolate-plant combinations producing both the greatest yield and the most rapid speed of kill. Differences between virus isolates primarily occurred because they responded to virus dose in different ways, with one isolate (Mandarte) producing a decreasing yield with increasing virus dose. The mechanisms behind these differences are as yet unclear: insects killed by higher virus doses usually die more rapidly and this might be expected to result in a lower virus yield. However, other studies have shown that virus yield is often increased at higher virus doses, despite being combined with a more rapid speed of kill (Cory *et al.* 2004). This underlines the importance and complexity of the dose–response relationship.

Overall, insects infected on alder produced more virus OBs (and had a higher cadaver weight). It is not clear why this might be the case. The insects used in the experiment originated from an alder population so they may perform better on alder. However, all insects in the experiment were reared predominantly on alder. Thus rearing effects would be expected to be the same across all treatments. It is possible that the short infection period on the different host plants may have influenced processes within the insect gut. For example, gut cells are shed as a response to virus infection and this is affected by host plant chemistry (see below). This process could vary between different virus–plant combinations and have lasting effects on gut integrity and food uptake. This is a complex issue and further experimentation is needed to tease apart potential mechanisms.

A link between rapid speed of kill and high yield has been found in another baculovirus system, the winter moth, *Operopthera brumata*, and its NPV. When the response of two geographic virus isolates on three host plants was compared, a significant negative relationship was found between the speed of kill and yield across each virus–plant combination (Raymond *et al.* 2002). Several important similarities exist between the western tent caterpillar and winter moth studies: firstly the virus isolates used in the bioassays had only undergone limited passage in the laboratory and utilized field collected insects (for *M. c. pluviale* the virus isolates were used directly from the field). They thus better reflect what is happening in the field situation than studies carried out on virus isolates that have been continually amplified in the laboratory in cultured insects. Secondly, the viruses used in the assays were not cloned. Field-collected NPV isolates, even those from single larvae, are likely to be comprised of mixtures of genotypic variants (Hodgson *et al.* 2001). NPVs isolated from *M. c. pluviale* are genetically variable. Genetic analysis of NPV isolates (single larvae) taken from the sites used in the current study in the same year, show that the proportions of dominant variants differ among the sites and not all variants are present at all sites (Cooper *et al.* 2003). Mixed genotype pathogen populations will be more adaptable and the current results indicate that a balance could exist in field populations between variants with different phenotypic traits such that both speed of kill and yield are optimized. Adaptation to local host plant

populations could result from selection among different virus genotypes that have variable phenotypic characteristics on different host plants. The phenotype of the constitutive individual virus variants has not yet been studied in *M. c. pluviale* NPV. However, single genotypes of pine beauty moth (*Panolis flammea* D & S) NPV have been demonstrated not only to have quantitative differences in key phenotypic traits (Hodgson *et al.* 2001), but also to show differential activity on different host plants for both pathogenicity and speed of kill (Hodgson *et al.* 2002, D.J. Hodgson, R. Hitchman, A.J. Vanbergen, R.S. Hails, R.D. Possee and J.S. Cory, unpublished data).

Virus pathogenicity was not apparently influenced by host plant, although pathogenicity has been shown to be significantly affected by plant species in other studies (e.g. Duffey *et al.* 1995; Ali *et al.* 2002) and individual *P. flammea* NPV genotypes did show differential pathogenicity on two host plants (Hodgson *et al.* 2002). Host susceptibility was variable in our assay and this might mask any more subtle differences in virus pathogenicity.

Two features are evident in this study. Firstly, the interaction between host plant chemistry and virus infection processes provides sufficient variety to influence selection on the virus. Secondly, some virus populations can gain higher productivity without any cost in terms of speed of kill and this may be due the presence of different virus variants. Robust empirical studies of mixed infections and their consequences for pathogen fitness are rare. However, preliminary evidence from studies with *P. flammea* NPV suggests that infections with two genotypes can have both enhanced pathogenicity and yield, in comparison with single genotype infections, with no increase in time to death (D.J. Hodgson, R. Hitchman, A.J. Vanbergen, R.S. Hails, R.D. Possee and J.S. Cory, unpublished data). The mechanism behind these results is as yet unclear, however it is possible that different variants may have slightly different tissue tropisms resulting in more rapid and widespread use of host tissues or cells. Alternatively, mutualistic interactions between co-infecting genotypes may occur, for example, via complementation in the action of different genes within the genetically diverse population (Frank 2003).

The mechanism for differential selection among virus genotypes is likely to centre on the processes that occur in the larval mid-gut. Much of the work on the chemical ecology of insect–baculovirus interaction has focussed on the mid-gut as the major barrier to infection (review in Cory & Myers 2003). A baculovirus must be ingested in order to initiate infection. In the mid-gut a combination of high pH and proteases (and possibly other virus-derived gene products) breaks down the OB to release the virus particles. The virus particles then invade through the peritrophic membrane and into the mid-gut cells from where they infect other tissues within the insect (Volkman 1997). Any compound that alters gut pH or the invasion of the peritrophic membrane will influence the breakdown of the OB and the infection process (Keating *et al.* 1989). One means of defence the insect possesses is to slough off infected gut cells before the infection can become systemic (Washburn *et al.* 1998). The virus particles therefore must initiate a systemic infection before they are removed. Host plant derived chemicals can interfere with these processes. Foliar oxidative enzymes, in particular peroxidases, inhibit baculovirus infection and reduce speed of kill (Hoover *et al.* 1998b) and this effect is modified by interactions with phenolics (Hoover *et al.* 1998a). One mechanism behind this inhibition appears to be that peroxidase activity (via the generation of free radicals) damages the insect gut, and increases sloughing of cells and thus the removal of virus-infected cells (Hoover *et al.* 2000). It has also been demonstrated that the diet of the insect host can alter the pathogenicity of virus produced by infected insects (MacDonald & Ritter 1988). Thus selective pressure on the virus could be strong at several points in the infection process. As yet, no baculovirus genes that interact specifically with plant components have been described, although numerous genes are known to influence virus infectivity and the function of many genes in the baculovirus genome are still unknown.

The tritrophic interaction between western tent caterpillars, NPV and three host plants underlines the importance of spatial variation in host–parasite interactions, and in particular, the potential influence of genotype × environment interactions for the pathogen as well as the host. While it is necessary to test the generality of this finding over different spatial and temporal scales and in other systems, these results indicate the complexity of interactions that determine selection on pathogen virulence in field populations.

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