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Within and between population variation in disease resistance in cyclic populations of western tent caterpillars: a test of the disease defence hypothesis

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Summary

1. Epizootics of nucleopolyhedrovirus (NPV) are an obvious component of the population fluctuations of several species of temperate forest Lepidoptera, including the western tent caterpillar, *Malacosoma californicum pluviale* (Dyer). An observed relationship between epizootics and the subsequent reduction in fecundity of populations led to the formulation of the disease defence hypothesis. This hypothesis predicts that viral epizootics in peak populations select for more resistant moths and that their reduced fecundity in declining populations reflects a cost of disease resistance.

2. To test the disease defence hypothesis, we carried out bioassays to measure the variation in larval resistance to NPV infection for families of western tent caterpillars from four spatially distinct populations over 3 years of peak and declining host densities.

3. Each female moth lays a single egg mass and larvae are gregarious and remain together through development. We found that the resistance to disease of larvae within families was not related to the number of eggs in the mass from which they hatched (the fecundity of their mother).

4. Disease resistance of larvae varied among populations and over time in a manner consistent with selection for resistance. One population that had not experienced a strong viral epizootic during the last population decline was more susceptible to infection in the first year of the study. Larvae from a second population that experienced an early epizootic became significantly more resistant. The resistance of two other populations increased slightly before the viral epizootic occurred in the field however, and thus could not be explained by selection.

5. As population densities declined from peak density, the background mortality of larvae increased and the fecundity of moths decreased. This indicates a general deterioration in the quality of field populations of tent caterpillars associated with the declining populations.

6. Although some evidence suggests that viral epizootics can select for increased resistance of field populations of tent caterpillars, the general deterioration in quality, elevated background mortality, and the reduced fecundity after the epizootic are stronger influences on the population decline. These are possibly related to sublethal viral infection.

Key-words: baculovirus, costs of resistance, heterogeneity, insect outbreaks, population cycles

Introduction

Macro- and microparasites affect the reproduction and/or survival of hosts, and thus they have the potential to influence host population dynamics. One of the most dramatic examples of the impact of disease on population dynamics are the epizootics of the microparasite nucleopolyhedrovirus (NPV) in peak and declining populations of forest caterpillars in the families Lasiocampidae and Lymantriidae (Otvos, Cunningham & Friskie 1987; Dwyer *et al.* 2000; Myers 2000; Van Frankenhuyzen *et al.* 2002; Cory & Myers 2003), and in some sawflies in the family Diprionidae (Entwistle *et al.* 1983; Moreau *et al.* 2006). Levels of viral infection in peak and declining populations can be very high, with over 75% of the population dying of infection in one generation. This high level of mortality, in addition to reducing population density,

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could be a strong selecting force on host populations. Thus for cyclic populations of forest caterpillars, viral epizootics have the potential to influence both the population and evolutionary dynamics of their hosts and co-evolutionary interactions between host resistance and pathogen virulence (Pimental 1968).

Variation in disease susceptibility among individuals is a prerequisite for pathogen epizootics to select for host resistance. Studies of genetic variation for parasite or pathogen resistance in natural populations of animals are limited. When it has been investigated however, significant levels of variation are often found (e.g. Ebert, Zschokke-Rohringer & Carius 1998; Webster & Woolhouse 1998). Examples of parasite-mediated selection in natural populations remain extremely rare (Little 2002; Duffy *et al*. 2008), and are mostly for small, easily reared, aquatic organisms.

Natural variation for resistance to baculoviruses (NPVs and granuloviruses) has been demonstrated in field populations of several lepidopteran species (Briese & Mende 1981; reviews in Watanabe 1987 & Fuxa 2004), although it has yet to receive much attention in an ecological or evolutionary context. Lepidoptera have been selected for resistance to viral infection in the laboratory (e.g. Briese & Mende 1983; Fuxa & Richter 1989; Abot *et al*. 1996; Milks & Myers 2000) and in the field (Asser-Kaiser *et al*. 2007). In some cases, a cost to resistance has been suggested (Fuxa & Richter 1998) and in other studies resistance has been stable without additional selection (Milks, Myers & Leptich 2002; Eberle & Jehle 2006). An early study by Martignoni (1957 cited in Watanabe 1987) reported that cyclic populations of the larch budmoth, *Zeiraphera diniana*, significantly increased in resistance to a granulovirus after a disease epizootic that caused up to 50% mortality. Subsequent cycles of the larch budmoth however continued without virus epizootics (Baltensweiler *et al*. 1977; Turchin *et al*. 2003), and interest in virus as a causative agent in this system has dwindled.

Many hypotheses have been proposed to explain the cyclic dynamics of forest caterpillars; induced plant defences, food limitation and crowding, maternal effects, parasitoid attack, and diseases (Myers 1988; Kendall *et al*. 2005). These hypotheses have received almost more attention from modellers than empirical study however (see references in Liu, Bonsall & Godfray 2007). Many mathematical models have been published that consider how the dynamics of viral transmission and host susceptibility might yield cyclic dynamics (partial review in Cory & Myers 2003; Dwyer, Dushoff & Lee 2004; Dwyer, Firestone & Stevens 2005). These models have not however, taken into consideration two important characteristics of declining populations of some forest Lepidoptera, the continued reduced fecundity of moths into the population trough and the potential for rapid evolution in the resistance of populations to infection.

In south-western British Columbia, western tent caterpillars, *Malacosoma californicum pluviale*, fluctuate in density, with peaks occurring approximately every 6 to 11 years (Myers 2000). Population outbreaks of this species are associated with epizootics of NPV disease, followed by a decline in the levels of infection in the subsequent generations to very low levels during population troughs. Fecundity is also lower during the decline (review in Myers 1988, 2000) and this provides the delayed density dependence required for cyclic population dynamics. Given the prevalence of NPV infection in this species, a fundamental question is whether reduced fecundity is associated with surviving the virus epizootic. This scenario led Myers (1988, 1990) to propose the 'disease defence hypothesis' (DDH) that predicts that viral epizootics select for more resistant individuals and that the reduced fecundity of moths reflects a cost of resistance to viral infection. This might result from a change in the physiology or morphology of larvae that reduces their susceptibility to virus, or the costs of mounting an immune response to infection. An alternative explanation is that reduced fecundity indicates a cost of surviving virus challenge e.g. a loss of resources to early viral infection, and/or the effect of continued sublethal (persistent) viral infection following the epizootic (review in Cory & Myers 2003). Thus far, evidence more strongly favours the latter explanation (Rothman & Myers 1994, 1996; Rothman 1997).

Requirements for the DDH are that genetic variation for susceptibility to disease must exist among families or individuals and changes in resistance to viral infection must occur in field populations after virus epizootics. Here we test these assumptions by monitoring the levels of resistance to NPV of field-collected families of western tent caterpillars over three generations, encompassing the periods of pre-peak, peak and initial population decline. We predicted that families of this gregarious species would differ in their levels of resistance to viral infection, and that families from populations that had recently experienced a viral epizootic would be the most resistant. We also predicted that, if the levels of resistance to infection are associated with fecundity, the size of the egg mass giving rise to more resistant families would be smaller (Rothman & Myers 1996).

Materials and methods

FIELD POPULATIONS

Western tent caterpillars occur near the western coast of North America from British Columbia to California. They feed on a variety of deciduous trees and shrubs, and live gregariously on and in conspicuous silk tents. Female moths lay a single egg mass of 150 to 300 eggs. Overwintered egg masses hatch in early April, larvae feed and then pupate in late May to early June, and the adults emerge in June and mate immediately.

We monitored population trends by counting the number of tents annually in specific sites and rearing a subsample of larvae from individual tents to estimate the level of NPV infection in the field as the proportion of families that demonstrated viral infection. In addition, we collected egg masses after larvae have hatched in the field and counted the number of eggs per mass to estimate the fecundity of moths as each female lays all of her eggs in one mass. For this study, we have monitored populations from four island locations: Mandarte Island, a 7-ha island in the Haro Strait south-east of Vancouver Island, Galiano and Saturna Islands in the Southern Gulf Islands, and on Westham Island in the delta of the Fraser River,

Table 1. The original number of *Malacosoma californicum pluviale* egg masses collected and the number of families that were eliminated because they had viral infection or other causes of mortality before the start of the assay or because of high levels of mortality in the controls during the assay

	Original egg masses	Removed for virus	Removed other deaths	Assayed	Eliminated for high control deaths
2003					
Galiano	10	0	0	10	0
Mandarte	5	0	0	5	0
Saturna	10	2	0	8	0
Westham	6	0	0	6	0
2004					
Galiano	11	0	0	11	6
Saturna	9	2	0	7	3
Westham	9	0	0	9	1
2005					
Galiano	8*	0	0	8	0†
Westham	8‡	0	5	3	0†

*In 2005, all egg masses from Galiano were derived from crosses of moths emerging from pupae collected from the field in 2004. †Mortality from other causes was very high in treated insects in all families. ‡Three egg masses from Westham were derived from crosses of moths from pupae collected in 2004 and five were field collected, of which three died from fungal infection.

south of Vancouver. These sites are described in more detail in Myers (1990, 2000).

PRE-PEAK POPULATIONS — 2003

In the early spring of 2003, we collected egg masses from four populations; Saturna, Galiano, Westham and Mandarte Islands (Table 1). The egg masses were kept at ambient temperature in a protected location outside until early April, and then moved to 4 °C to prevent them from hatching. The egg masses were then divided into three groups as it was not possible to bioassay them all simultaneously. Each group was kept at a different temperature between 4 °C and ambient for a short period in order to stagger their hatching slightly over a period that roughly corresponded to the normal hatching time for the western tent caterpillar in the field. All sites were represented in each group and the timing of all the bioassays overlapped. All egg masses were surface sterilized by immersing them for 5 min in 0·8% bleach solution to remove the spumaline coating and external contamination by pathogens. Each family was reared individually in 1 L waxed paper cups and was provided with surface-sterilized red alder, *Alnus rubra* Bong. leaves *ad libitum*. Any families that showed evidence of disease during rearing were removed from the study (Table 1). The number of eggs in each egg mass was counted after the larvae had hatched.

PEAK DENSITY POPULATIONS — 2004

Egg mass collection was not feasible in this year from the more inaccessible Mandarte Island but was from the other three sites in 2004 (Table 1). Egg masses were kept cool in the same conditions until use in the bioassay in late April/early May and then reared in family groups as before. The number of eggs per mass was counted after the larvae hatched for most families although several egg masses were accidentally discarded.

POST-VIRUS EPIZOOTIC — 2005

As all the populations suffered high levels of NPV mortality in the field in 2004 (Fig. 1), we knew that it would be difficult to collect egg masses, and thus we collected pupae from Westham and Galiano in late June 2004. No pupae could be found at the Saturna site. Pupae were returned to the laboratory and left to emerge as adults. Pairs of moths were placed in a 500 mL paper cup with a freshly cut alder twig and left overnight. Egg masses were usually successfully produced from pairs after 24 h or 48 h. Egg masses were kept in a container in

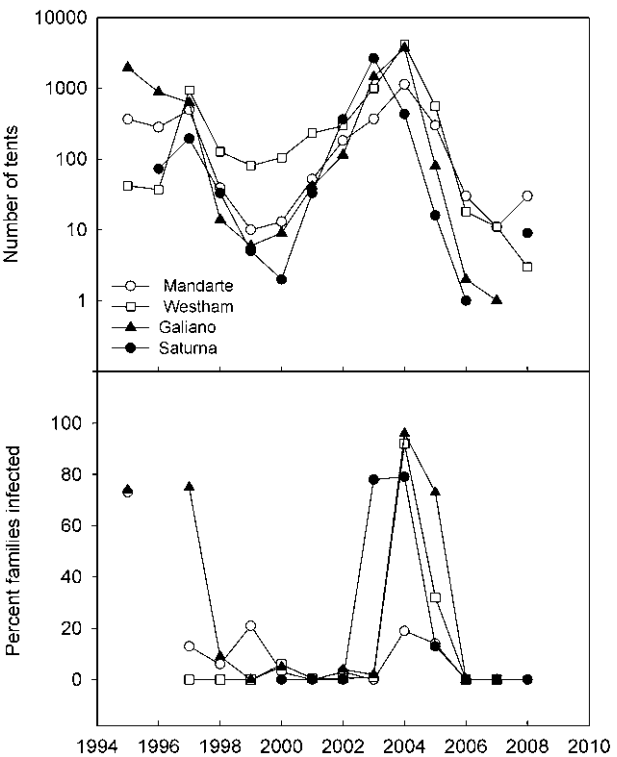

Fig. 1. Number of tents per monitoring site (top) and NPV infection level (bottom) in western tent caterpillar, *Malacosoma californicum pluviale*, populations at four sites in British Columbia over the most recent population cycle.

a protected location outside until early April, after which they were stored at 4 °C. Searches for egg masses were also carried out at the field sites in February 2005. No eggs were found at the Saturna or Galiano sites, but five were collected from Westham. Final numbers are given in Table 1.

VIRUS STOCK

Nucleopolyhedroviruses are transmitted among larvae following the death of an infected host and the release of virus occlusion bodies (OB); proteinaceous polyhedra approximately 1–5 μm in diameter containing virus particles. Concentrations of NPVs are determined by counting serial dilutions of OBs using a haemocytometer.

The McplNPV stock used in the bioassays originated from the Mandarte population in 1999. Population density and virus infection levels were very low at this time (Fig. 1). Two infected larvae from the same family were macerated individually in 300 μL of distilled water and equal volumes of each were mixed. Ten microlitres of this solution was painted onto several decontaminated alder leaves to which groups of five fourth instar tent caterpillars were added and reared through until death. Virus was extracted by macerating the virus-killed larvae in milli Q water on ice, filtering through muslin and pelleting for 30 min at 3500 g. After two washes with water, the resuspended pellet was centrifuged at 70 000 g at 4 °C for 90 min on a discontinuous 50–60% (w/w) sucrose gradient. The virus was harvested, washed and pelleted three times in sterile distilled water at 3500 g. Virus concentration was estimated by 10 counts of a 1 in 100 dilution using a Neubauer improved haemocytometer (B.S. 748, Weber, Teddington, England). The virus was stored at −20 °C in 500 μL aliquots.

BIOASSAYS

The larvae were reared in their family groups until the fourth instar. The number of larvae in each family was then counted and the larvae were divided into six equal-sized groups, one for each dose treatment plus the control. Larvae that had moulted to the fourth instar in the same 24-h period were set up in the bioassay and, for most families, bioassays had to be set up on two consecutive days to account for variation in time to moult. Larvae were then infected individually on small discs of alder leaves (8 mm diam.) with either distilled water or one of five NPV doses. The McplNPV stock was serially diluted in distilled water so that doses of OBs could be administered to the larvae in 3 μL of distilled water. In 2003, for the first two bioassay groups the dose range was 45 000, 22500, 11 250, 5625 and 1407 virus OBs per larva. For the third group, the lower dose was dropped and replaced by a higher dose of 90 000 OBs per larva in order to obtain higher mortality levels. As virus dose can influence other parameters, such as speed of kill, we decided to return to the initial dose range in 2004 and 2005. This did not affect the virus dose-insect mortality analysis, but it did mean that summary statistics, such as mean mortality, used to compare among families and years, had to be averaged over a reduced dose range to make the comparisons equivalent. In each year, insects were infected using a fresh aliquot of the same virus isolate. Insects were left for 24 h and those that had eaten the leaf disc were transferred to fresh foliage. Where possible, up to 30 larvae were set up per dose treatment. Insects were reared at room temperature with a natural light cycle, fed on red alder as needed (usually every other day) and monitored daily for 15 days. Larvae that had been killed by virus usually displayed the distinctive characteristics of NPV infection (thin, easily ruptured cuticle and oozing, milky liquid), but cause of death was confirmed using phase-contrast light microscopy when diagnosis was ambiguous.

CONSTRAINTS ON SAMPLE SIZES

Using field populations creates several constraints on the number of families that were compared in this study of variation in resistance. In 2003, the number of families tested was limited by the fact that bioassays had to be carried out over a period of 2 months corresponding to the time of development of larvae and their food plants in the field, as well as by the number of families that could be inoculated at one time. Natural declines of populations in the field by 2004 and 2005 reduced the number of egg masses that could be collected. In addition, families had to be removed from the analysis because they showed signs of infection before the bioassays started or the control groups had high levels of viral infection or other mortality (Table 1). We do not know the impact of removing families with high background mortality from the assay, but have assumed that their removal reflects the natural selection that would have occurred in the field.

STATISTICAL ANALYSIS

In order to compare disease resistance within and among populations (sites) in each year, the proportion of virus-induced mortality was analysed using generalized linear models with a binomial distribution and a logit link structure (JMP 6, SAS Institute). Site and family nested within site, with $\log_{10}$ virus dose as a covariate, and their interactions were included as explanatory variables in the maximal model. The contribution of each explanatory term was then tested sequentially, starting with the highest-order interactions and nonsignificant terms removed from the model to produce the minimal model following standard stepwise deletion protocols (e.g. Crawley 2002). The data showed a low level of overdispersion, thus a scale parameter (Pearson's chi-squared/degrees of freedom) was used and F values were calculated; this procedure does not change the parameter estimates but increases the standard errors. In 2004, natural virus mortality in the untreated controls was high in some families and was accounted for before analysis; the adjusted sample size n^* at each virus dose was taken to be $n^* = n(1 - x)$, where n is the original sample size and x is the virus mortality in the untreated control, and if y insects responded, the adjusted $y^* = y - nx$, such that y^*/n^* is used as the relationship for modelling the logit response to virus dose (Collett 2003). Nonviral mortality from other causes was combined and analysed separately, again accounting for background nonviral deaths in the controls. In order to lose as little data as possible, families with up to 28% background virus mortality or 30% natural mortality from other causes were included in the analysis, although the majority of families showed 10% or less background mortality, and several had none at all. Additional analyses were carried out using general linear models. Data were first checked for normality and transformed if necessary.

Results

The recent fluctuations in the western tent caterpillar populations are shown in Fig. 1. The population outbreak at the Saturna Island site preceded those on the other islands by a year (peak in 2003 versus 2004) (Fig. 1). Egg masses that gave rise to the larvae for the first year of our study of disease resistance were laid in 2002 and collected in the early spring of 2003. They thus represent the larvae that made up the peak

population on Saturna Island, but the pre-peak populations for the other three sites in 2003. By 2004, the Saturna population had begun to decline while the other populations were at peak density, and by 2005 all populations were in the decline phase.

Levels of infection by *M. c. pluviale* NPV (McplNPV) in these populations are highest at peak density (Fig. 1). Levels of NPV infection in all populations were very low in 2002 and therefore, populations tested for resistance to virus in 2003 would not have been selected by high levels of infection since the last outbreak in 1995 (Myers 2000). For 2004, families from Saturna Island would have represented moths that had survived a virus epizootic as larvae in 2003, but those from Galiano and Westham would not have been selected in an environment of high viral infection. By 2005, all populations had been exposed to the high levels of viral infection that had occurred in the previous year.

VARIATION IN DISEASE RESISTANCE WITHIN AND AMONG POPULATIONS

Pre-peak populations 2003

As expected, increasing virus dose significantly increased the resulting level of virus-induced mortality in the insects ($\log_{10}$ dose: $F_{1,115} = 360{\cdot}15$, $P < 0{\cdot}0001$). Larval resistance varied among sites (site: $F_{3,115} = 13{\cdot}53$, $P = 0{\cdot}0036$) (Fig. 2a). Insects from Westham Island were significantly more susceptible to fatal NPV infection than insects from the other three populations (contrasts; Westham v Galiano, Mandarte and Saturna, $\chi^2 = 10{\cdot}52$, $P = 0{\cdot}0012$). The largest differences in susceptibility to NPV were between the different families of tent caterpillar within each site [family(site): $F_{25,115} = 252{\cdot}64$, $P < 0{\cdot}0001$] (Fig. 3). All populations responded similarly to changes in dose level ($\log_{10}$ dose × site: $F_{3,87} = 2{\cdot}125$; $P > 0{\cdot}05$). Virus mortality in the controls was negligible and each site responded similarly in each group of assays (site × group: $F_{6,132} = 11{\cdot}79$, $P = 0{\cdot}07$).

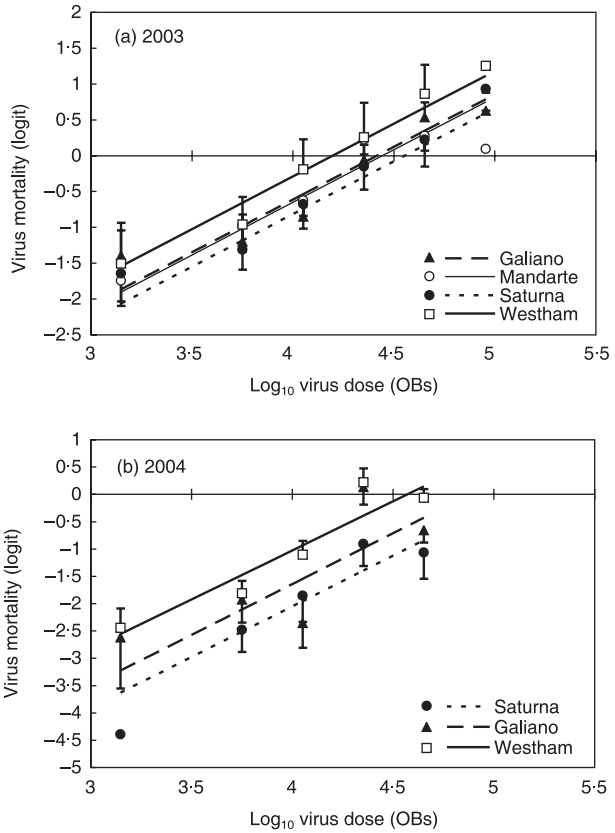

Fig. 2. Susceptibility of western tent caterpillar populations to McplNPV in (a) 2003 and (b) 2004. Lines show the fitted statistical model and points are the average mortality per family in each site ± standard errors ($n = 10$, 5, 8 and 6 in 2003, for Galiano, Mandarte, Saturna and Westham and $n = 5$, 4 and 8 in 2004 for Galiano, Saturna and Westham respectively). Fitted model lines are presented without the inclusion of family (site) for clarity. Lines are; (a) logit virus mortality = 1·4689 $\log_{10}$ virus dose minus i) 6·4881, ii) 6·5274, iii) 6·6881 and iv) 6·1648 for Galiano, Mandarte, Saturna and Westham respectively, and (b) logit virus mortality = 1·8 $\log_{10}$ virus dose minus i) 8·5839, ii) 8·898 and iii) 8·2091 for Galiano, Saturna and Westham respectively.

Peak populations 2004

As found in 2003, virus-induced mortality increased in the families with increasing virus dose ($\log_{10}$ dose: $F_{1,67} = 104{\cdot}85$; $P < 0{\cdot}0001$), and all populations responded similarly to changes in dose level ($\log_{10}$ dose × site: $F_{2,51} = 0{\cdot}004$; $P > 0{\cdot}05$). Larval susceptibility varied between populations (site: $F_{2,67} = 16{\cdot}72$; $P = 0{\cdot}0002$) (Fig. 2b), with Saturna being the most resistant population (Saturna vs. Galiano and Westham: $\chi^2 = 11{\cdot}79$, $P < 0{\cdot}001$), although the order of response, in terms of increasing susceptibility to virus, remained the same as in 2003. Susceptibility among different families within the sites also varied significantly [family(site): $F_{14,67} = 29{\cdot}26$; $P = 0{\cdot}01$] but less so than in the previous year (Fig. 4).

We then tested to see if the background mortality not attributable to virus infection was influenced by virus treatment in the families retained in the bioassay analysis in 2004. It was not possible to use exactly the same family data set as several families had no nonviral deaths (four families; two from Galiano, one each from Saturna and Westham Islands). After removing these, there was a weak relationship with virus dose, with nonviral deaths showing a slight increase with increasing virus dose ($\log_{10}$ dose: $F_{1,68} = 5{\cdot}38$, $P = 0{\cdot}02$). However, there was no difference in nonviral mortality among sites or families [site: $F_{2,55} = 0{\cdot}79$, $P > 0{\cdot}05$; family(site): $F_{11,55} = 12{\cdot}02$, $P > 0{\cdot}05$].

Post-epizootic populations 2005

The initial survival in family groups was high in 2005 until the larvae were separated for the bioassay at the fourth instar. The survival of the water-treated control families was high after separation (98% Galiano and 92% Westham), but in the remaining families, 55% of larvae died from unidentified causes within 48 h (most in the first 24 h) following challenge

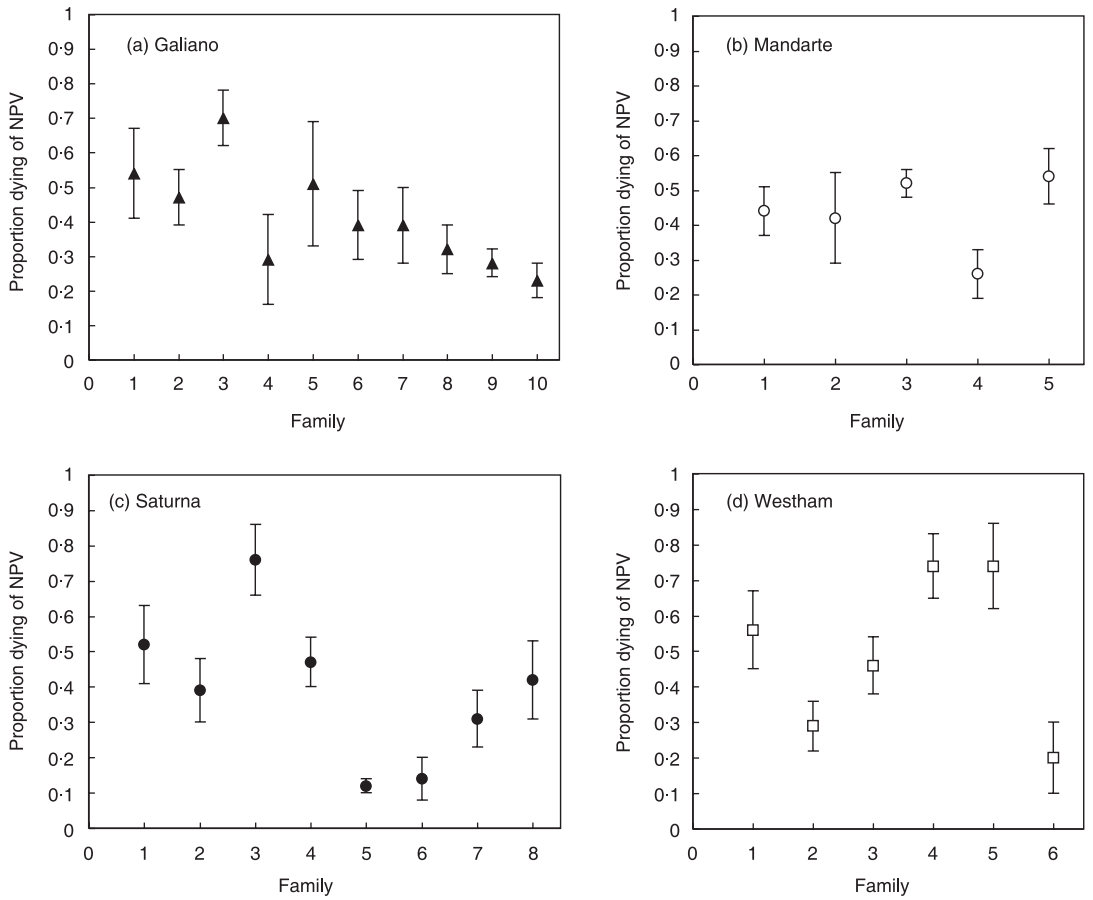

Fig. 3. Average proportion (± s.e.) of tent caterpillar larvae per family dying following exposure to McplNPV in assays carried out in 2003: a) Galiano, b) Mandarte, c) Saturna and d) Westham. Points show the average response across virus doses of 5625, 11 250, 22 500 and 45 000 OBs per fourth instar larva; the highest and lowest doses have been omitted as they were not constant across all families.

with virus. Insufficient insects remained to carry out a dose response analysis. The percentage of larvae dying from viral infections averaged across the four highest doses was Westham 20% (± se 2, $n = 8$ families) and Galiano 13% (± se 3, $n = 3$ families). These levels of viral deaths appear to be lower than those in 2003 and 2004 (Figs 3–5) but the high background mortality makes statistical comparison inappropriate and conclusions tentative. Again, Westham families tend to be more susceptible than Galiano families.

THE RELATIONSHIP BETWEEN FECUNDITY AND RESISTANCE TO VIRUS

Egg mass size of the insects used in the 2003 bioassays (produced by the 2002 generation) ranged from 144 to 331 and the mean egg mass size did not vary among sites ($F_{3,22} = 0{\cdot}5072$, $P > 0{\cdot}05$). Virus mortality in the bioassay was not correlated with the size of the egg mass ($F_{1,24} = 0{\cdot}1094$, $P > 0{\cdot}05$, Fig. 5).

The number of eggs per mass in 2004 (eggs laid in 2003) did not vary among sites either when comparing all the egg masses set up for the bioassays or just those families that were used in the final bioassay analysis ($F_{2,22} = 0{\cdot}0092$, $P > 0{\cdot}05$ and $F_{2,13} = 0{\cdot}3218$, $P > 0{\cdot}05$, respectively). Egg mass size was not related to the proportion of virus deaths in the bioassay ($F_{1,14} = 0{\cdot}1672$, $P > 0{\cdot}05$, Fig. 5). The egg mass sizes of those families included and excluded from bioassay analysis did not differ ($F_{1,23} = 0{\cdot}0331$, $P > 0{\cdot}05$; 203 (± se 6) included and 206 (± se 17) excluded). Eggs per mass were not counted in the 2005 bioassay but field collected egg masses were 2 to 25% smaller that year than in 2004 (Fig. 6).

Discussion

In this study, we tested the 'disease defence hypothesis' as an explanation for the cyclic dynamics of western tent caterpillars and potentially other species of forest Lepidoptera for which viral epizootics are associated with cyclic dynamics. A consistent characteristic of declining tent caterpillar populations is reduced fecundity of moths; each female lays a single egg mass (Myers 2000). This might be explained by either the costs of resistance to virus (DDH) or as a sublethal effect of larvae surviving virus challenge. The DDH predicts the existence of (i) genetic variation for virus resistance within tent caterpillar populations, (ii) a trade-off between fecundity and disease resistance, and (iii) that disease resistance within a population will increase following a virus epizootic with a correlated decrease in fecundity.

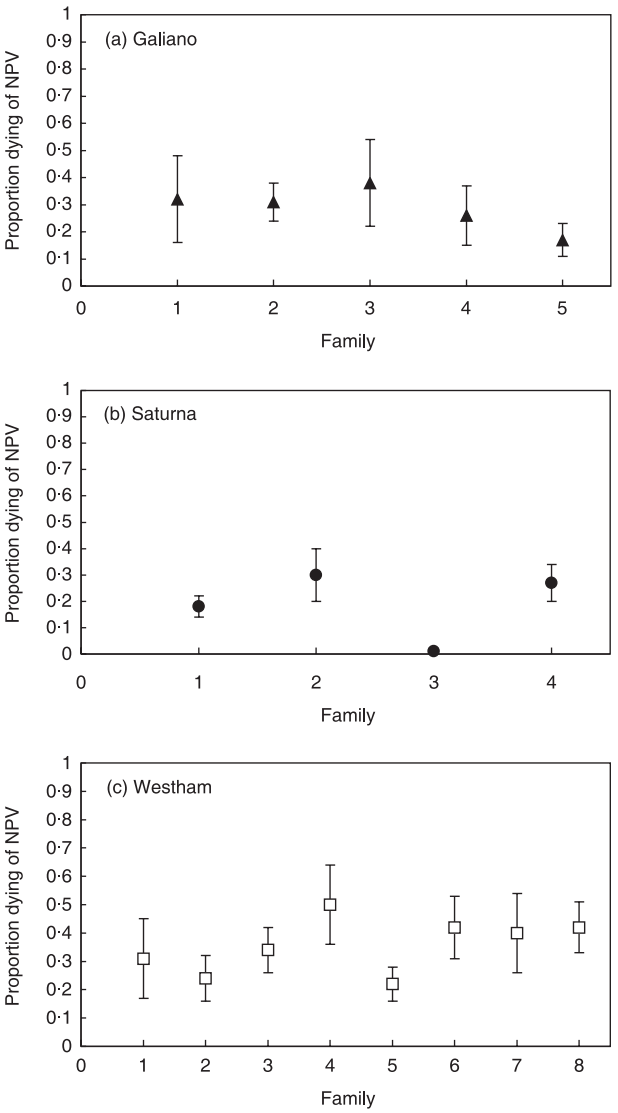

Fig. 4. Average proportion (± s.e.) of tent caterpillar larvae per family dying (adjusted for control mortality) following exposure to McplNPV in assays carried out in 2004; a) Galiano, b) Saturna, and c) Westham. Points show the average response across virus doses of 5625, 11 250, 22 500 and 45 000 OBs per fourth instar larva; the lowest dose has been omitted in order to compare the values with 2003.

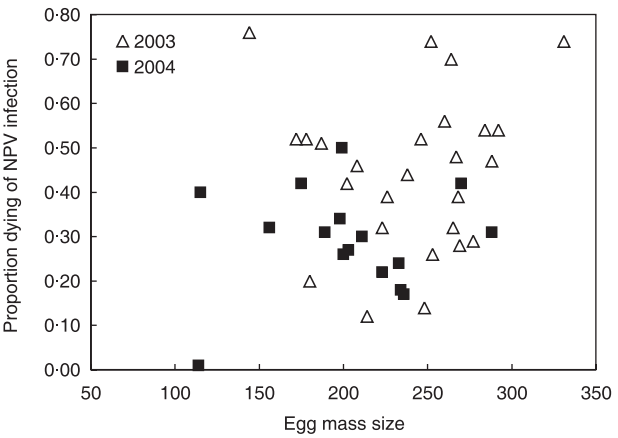

Fig. 5. Relationship between egg mass size (moth fecundity) and the proportion of larvae killed averaged across McplNPV doses 5625, 11 250, 22 500 and 45 000 OBs per fourth instar larva (a measure of resistance) in 2003 and 2004. In neither year was the relationship significant (see text). Egg masses of the families bioassayed were slightly larger in 2003; average of 242 (± 10·5 s.e.) eggs per mass in 2003 compared to 203 (± 12) eggs in 2004 ($F_{1,34} = 6{\cdot}1108$, $P = 0{\cdot}0186$). The proportion of insects dying of virus infection in the bioassays decreased from 2003 to 2004 ($F_{1,178} = 24{\cdot}84$, $P < 0{\cdot}0001$).

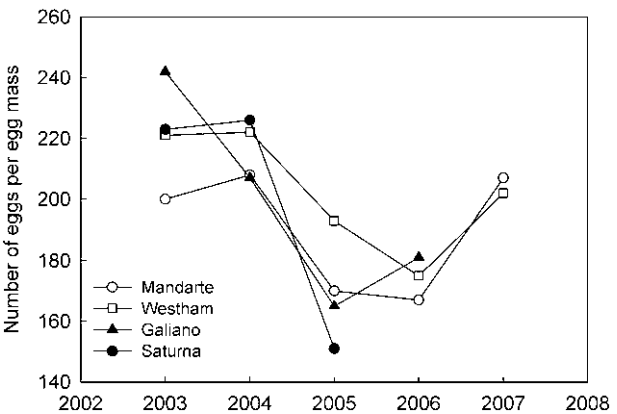

Fig. 6. Average number of eggs per egg mass during the declines in *Malacosoma californicum pluviale* population density that began between 2003 and 2004 for the Saturna population and between 2004 and 2005 for the other three tent caterpillar populations (Fig. 1). Egg masses are collected in the spring of the year and represent those of female moths that developed as larvae in the previous year.

The first of these predictions is supported by the observed variation for resistance to baculoviral infection among families suggesting that sufficient heterogeneity exists for selection to act. Whether the variation in resistance has a genetic basis is not known, but several laboratory studies on other insect-baculovirus systems have demonstrated that resistance to baculoviruses can be heritable (e.g. Watanabe 1987; Asser-Kaiser *et al.* 2007). Heterogeneities in the probability of infection, at either the population or the individual level, will affect disease transmission and thus, potentially, population dynamics (Dwyer, Elkinton & Buonaccorsi 1997; Fenton *et al*. 2002).

The second prediction of DDH is rejected however, as no linear relationship between egg mass size (fecundity) and the susceptibility of families to virus infection occurred in either year studied. The fecundities of all populations were slightly lower in 2004 and this could have been associated with crowding of populations the previous year.

We conclude that the reduction of egg mass size in declining populations of tent caterpillars is not strongly related to changes in resistance to viral disease. Sublethal, persistent virus infection could explain the reduced fecundity indicated by small egg masses and this needs further investigation. In

the past, we have seen weak trends for larvae from small egg masses to be slightly more susceptible to virus and this is consistent with sublethal viral infection and vertical transmission of virus (Rothman & Myers 1996). It is most likely however, that the reduction in fecundity indicates a deterioration in the quality of populations with the beginning of the decline as indicated by the poor survival of larvae in the assays.

The outcome for the third prediction of DDH was more varied, but also potentially more revealing. The asynchrony of the virus epizootics in the field populations allowed us to compare populations that had and had not experienced an NPV epizootic. We could therefore examine whether this resulted in relative differences in resistance to disease and to separate the effect of a virus epizootic from other (unidentified) temporal trends in the population cycle that might also alter disease resistance. First, the lack of an apparent viral epizootic in the last outbreak in the Westham population and its higher density after the last decline, suggested relaxed selection there (Fig. 1). Thus, the greater susceptibility observed for this population, compared to the other sites in 2003, with a similar trend in 2004 and 2005, supports this prediction.

Second, the early virus epizootic in the Saturna population should have selected for increased resistance to NPV among larvae in 2004. Bioassay data support this prediction. A contradiction however, was the apparent increased resistance of the Westham and Galiano populations as well in 2004, before viral epizootics in these populations.

Finally, the viral epizootics in the Westham and Galiano populations in 2004 are predicted to have selected for increased resistance to virus in families assayed in 2005. Although bioassays in 2005 were compromised by very high levels of unexplained mortality, the proportion of larvae dying of virus in that year appeared to be lower than in previous years possibly indicating greater resistance.

The most striking feature of our study was the declining condition of the field populations over the three years of experiments. The same procedures were used in the bioassays in all 3 years; we are therefore confident that the differences between the assays in different years are related to the changing condition of the larvae, rather than contamination introduced during the bioassay process. In 2003, insects had extremely good survival in the laboratory, except for two families from Saturna that succumbed to NPV infection, a fate echoed by the field population in that year.

In 2004, the story was very different; a few families were lost before the start of the assay but the background mortality in the laboratory assays was very high in some families from all sites. This was despite the fact that little viral mortality occurred in the field at Galiano and Westham the previous year and that 2004 was the year of the viral epizootic in those populations. Mortality was also not solely due to virus infection, with fungal infection and several unidentified conditions killing the larvae. High levels of mortality in the untreated control insects in 2004 strongly indicate that it was unrelated to virus treatment, although it might have been exacerbated by it. The most likely explanation for the background mortality is covert infection by one or more pathogens that have been triggered or stressed into an active state.

The pattern of mortality in the bioassays in 2005 was interesting and different again. Although larvae survived well in the laboratory in family groups until the fourth instar, many insects succumbed to unknown causes of death 24 to 48 h after separation and exposure to the virus. This mortality was much too early for normal viral infection to have progressed and was not observed in the control groups. Most of the egg masses used in 2005 resulted from mating of adults that emerged from pupae collected from the field in the previous year and set up to mate in the laboratory. Thus, the chance of the eggs being externally contaminated from the field was negligible.

In the field, the sudden appearance of high levels of infection in 2003 (Saturna) and 2004 (Westham and Galiano) suggests a triggering of vertically transmitted virus in early instars followed by horizontal transmission among families in later instars. Baculoviruses and other insect pathogens can be transmitted vertically from adults to their offspring (review for NPVs in Kukan 1999; Burden *et al*. 2002). External contamination of the eggs (transovum transmission) is just one route of vertical transmission and pathogens can also be transmitted inside the eggs (transovarial transmission). All our egg masses and alder leaves were surface-sterilized which should remove transovum transmission of NPV, indicating that infection was likely to be transmitted within the egg. Transovarial vertical transmission is poorly understood, but evidence exists that baculoviruses can persist in a sublethal (persistent) form, that is symptomless, and does not result in an overt, lethal infection unless it is triggered in some way (Cory & Myers 2003). Increasing evidence suggests that such persistent infections can be maintained at high levels, without apparent cost, from one generation to the next (Burden *et al*. 2002, 2003).

Experiments by Beisner & Myers (2000) have shown that low doses of virus caused higher than predicted levels of infection within family groups of tent caterpillars in the field. As family sizes increased, so did the movement of larvae and the transmission of virus among family groups. In a gregarious insect, such as the western tent caterpillar, within-family density will be high enough for virus infection in early instars to be horizontally transmitted to susceptible later instars. This can result in several rounds of NPV transmission within a generation. When population densities increase, as they did between 2002 and 2004 (Fig. 1), family groups will intermingle, NPV infection will spread through the population and an epizootic will result. In this situation, sublethal infection can occur when late instar larvae are infected and still survive (Rothman & Myers 1996; Rothman 1997). This could lead to the substantial decline in fecundity that characterizes populations late in the decline phase (Myers 2000). The importance of the decline in insect condition and the decrease in egg mass size, as we have observed (Fig. 6), is that it could provide the delayed density-dependent factors necessary for cyclic population dynamics.

In conclusion, our studies show the following: (i) that the levels of resistance to virus infection can vary among families,

populations and generations of western tent caterpillars, (ii) that NPV can be transmitted vertically with eggs from which external contamination is removed, (iii) that eggs from parents that developed in conditions of low overt NPV infection (little opportunity for environmental contamination) can develop overt infection in the laboratory in the year of the initial epizootic in the field, and finally, (iv) a general deterioration of the condition of larvae from declining populations occurs and, although mortality from many causes is common, this may be accentuated by exposure to virus. As discussed by Beldomenico *et al*. (2008), pathogens may play an important role in an 'interactive web', as much as direct causes of mortality. In the end, we reject the DDH because on a family basis, the level of resistance was not related to the size of the egg mass, thus if this variation in resistance has a genetic basis, a cost of reduced fecundity is not indicated.

In terms of the dynamics of populations and patterns of infection, we are left with three important questions: (i) what maintains the high level of variation in resistance to virus in the field populations? (ii) What is the origin of the NPV that causes rapid increases in infection of field populations and in laboratory reared larvae from surface-sterilized egg masses? The lack of overt infection in the parental generation in the field makes this most curious. And (iii), what is the cause of the general decline in the quality of larvae from egg masses collected from peak populations? Explaining the population dynamics of forest caterpillars is not straightforward, and although viral infection and epizootics can play important roles for some species, other factors influencing the survival and fecundity of forest Lepidoptera are influential and cannot be ignored.

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