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Pathogen clumping: an explanation for non-linear transmission of an insect virus

VINCENT D'AMICO[1,2], JOSEPH S. ELKINTON[1], JOHN D. PODGWAITE[3], J. P. BUONACCORSI[4] and GREG DWYER[5] [1]University of Massachusetts at Amherst, Department of Entomology, U.S.A., [2]USDA Forest Service, University of Delaware, Townsend Hall, U.S.A., [3]USDA Forest Service, North-eastern Research Station, U.S.A., [4]University of Massachusetts at Amherst, Department of Mathematics and Statistics, U.S.A. and [5]University of Chicago, Department of Ecology and Evolution, U.S.A.

Abstract. 1. Previous work has shown that transmission of some insect pathogens is a non-linear process. A number of hypotheses have been put forward as explanations for this phenomenon; however, none have proven wholly satisfactory. Here we test the effects on transmission of spatial distribution of an insect virus by testing whether or not experimental manipulations of pathogen clumping lead to different values of a clumping parameter. The gypsy moth nucleopolyhedrovirus (LdMNPV) was used, which is transmitted when larvae consume virus released from previously infected larvae that have died on foliage.

2. It was found that even when virus densities on foliage were equal, overall mortality was lower when virus-killed cadavers were clumped on foliage.

3. Non-linearity is more pronounced when cadavers are clumped than when they are placed at random on the foliage. Placement of droplets containing LdMNPV on foliage resulted in more linear transmission compared with cadavers.

4. Spatial clumping of viral inoculum thus provides part of the explanation for non-linear transmission in this system. The ultimate explanation for non-linear transmission is likely to involve some combination of spatial clumping and heterogeneity in behaviours such as feeding rate or the ability to avoid pathogen.

Key words. Baculovirus, host–pathogen interaction, non-linear transmission.

Introduction

Both theory and data have suggested that horizontal transmission plays a key role in the dynamics of infectious diseases (McCallum *et al*., 2001). In studies of the ecology of baculoviruses, fatal, directly transmitted diseases of arthropods (Cory *et al*., 1997), this importance has become clearer partly through efforts to test mathematical models of disease. Much attention has focused on the so-called 'mass-action' assumption typical of such models (McCallum *et al*., 2001). For baculoviruses, which are transmitted when hosts accidentally consume infectious virus particles, this assumption is often written as

$$\frac{dS}{dt} = -vSP. \quad (1)$$

Here S is the density of uninfected hosts, t represents time and P is the density of infectious virus particles in the environment. The transmission coefficient v is assumed to be a constant, and dS/dt is thus linearly related to both S and P. Because this assumption has important effects on the behaviour of the models, such models are sometimes known as 'linear transmission' models. Although for many baculoviruses, there is good empirical evidence that this assumption is incorrect (Dwyer, 1991; Knell *et al*., 1998), there is only modest evidence in support of particular alternative models (but see Hails *et al*., 1997 and Dwyer *et al*., 1997).

Correspondence: Vincent D'Amico, University of Delaware, Entomology and Wildlife Ecology, Townsend Hall, Newark, DE 19717, U.S.A. E-mail: vdamico@fs.fed.us

In previous work, it was shown that an epidemic model based on equation 1 does not adequately describe the intensity of epidemics of nucleopolyhedrovirus in naturally occurring gypsy moth populations (Dwyer & Elkinton, 1993), which suggested that equation 1 does not apply to the transmission of this virus. In subsequent work various possible explanations for the failure of this model have been reported. Specifically, it was shown that equation 1 does not apply in small-scale experiments (D'Amico *et al*., 1996), suggesting that the failure of the model to explain epidemics has to do with transmission at a small scale. Additional small-scale experiments showed first that the failure of the model is *not* due to changes in per capita transmission with changes in the density of uninfected larvae S, and second that transmission does not change with changes in defoliation levels (D'Amico *et al*., 1998). The transmission rate of the virus, however, *does* change with virus density, and the extent of the change depends in turn on the host's population of origin (Dwyer *et al*., 1997; G. Dwyer, unpubl. data). These latter effects are consistent with epidemic models that incorporate host heterogeneity in susceptibility, suggesting that such host heterogeneity may explain non-linear transmission in this and other baculoviruses. Host heterogeneity in susceptibility, however, may be only one of several possible explanations for non-linear transmission (D'Amico *et al*., 1996; Dwyer *et al*., 1997). In this article, data are presented suggesting that another possible explanation is small scale spatial patchiness in the distribution of the virus on foliage.

The gypsy moth nucleopolyhedrovirus (also called LdNPV) is transmitted by ingestion of infective virions encapsulated within proteinaceous occlusion bodies, typically while caterpillars are eating foliage. After an infection is initiated larvae die within 2 or 3 weeks. The bodies of virus-killed larvae are fragile sacks of occlusion bodies and liquid, which rupture and spread their contents on foliage, or, in the case of later instars, the bole of the host tree (Cory *et al*., 1997). The virus thus deposited on foliage supplies inoculum for within-season transmission; several waves of virus mortality occur during the larval season, which occurs from mid-May to early July (Woods & Elkinton, 1987). The defoliation occurring during this time, particularly during high-density larval outbreaks, intensifies the concentration of virus on the foliage.

Between-year transmission occurs via a different process; egg masses laid by female moths in the summer are contaminated with virus physically scraped off the tree bole during oviposition (Murray & Elkinton, 1989, 1990). The virus overwinters on the egg mass, and is ingested by hatching larvae as they emerge in the spring. These early larvae disperse on the wind via ballooning. After the first instar, larval movement is greatly reduced, which means that the initial spatial distribution of virus changes only slightly as the season progresses.

Clearly, spatial clumping is a pronounced feature of the within-season transmission of nucleopolyhedroviruses in insects such as the gypsy moth, because larvae become infected by feeding on foliage contaminated with virus particles deposited when other larvae die from the virus. Because larvae are small relative to the area of leaves of most gypsy moth host plants, and because each infectious cadaver consists of a large number of infectious doses, the virus is very clumped on the foliage. Occlusion bodies may spread across the foliage slightly, particularly when it rains (D'Amico & Elkinton, 1995), but generally they remain highly concentrated.

Materials and methods

Mathematical models of the gypsy moth–virus interaction

To understand the consequences of natural history for epidemics of the virus, mathematical disease models can be used. This original model of the dynamics of the gypsy moth virus (Dwyer & Elkinton, 1993) provides a useful starting point for describing the model tested here. That model is

$$\frac{dS}{dt} = -vSP, \tag{2}$$

$$\frac{dI}{dt} = vSP - vS(t-\tau)P(t-\tau), \tag{3}$$

$$\frac{dP}{dt} = -vSP - \mu P, \tag{4}$$

where S, I, and P are the densities of uninfected insects, infected insects, and infectious cadavers respectively, v is the transmission parameter, τ is the time between infection and death, and μ is the rate at which the occlusion bodies in infectious cadavers are broken down. Two important features of this model are that, first, it assumes that transmission is linear, as described in the Introduction. Second, it ignores spatial structure of any kind. Because hatching gypsy moth larvae disperse long distances, it is suspected that virus populations in naturally occurring gypsy moth populations have little large-scale spatial structure, although the relevant data are lacking. The argument here, however, is that a key element that is missing in this model is small-scale spatial structure. This spatial structure arises as a consequence of the fact that infectious virus particles come in units of whole cadavers that are made up of vast numbers of occlusion bodies, so that spatial structure is manifest at the scale of leaves or portions of leaves. Simultaneously, however, space in such situations is clearly continuous, so that metapopulation models are not correct either. Modelling this kind of spatial structure requires either detailed simulations or mathematical approximation techniques that are technically complex (Bolker & Pacala, 1999; Lewis & Pacala, 2001). Because no such models are available for insect–virus interactions, the host–parasitoid modelling literature is used instead. Application of host–parasitoid models to insect–baculovirus interactions is not straightforward, for several reasons (Bonsall, 2004; Boots, 2004). For one reason, in baculovirus epidemics, there are potentially several generations of

the pathogen per host generation and the pathogen slowly breaks down, whereas in most host–parasitoid models there is only one parasitoid generation per host generation and the parasitoids do not experience mortality during the period while they are attacking the hosts. Host–parasitoid models can still be applied to this experimental data because in these experiments there is only one generation of the virus, and because the bags prevent the death of the virus.

The host–parasitoid model applied to these data assumes that the frequency distribution of parasitoid attacks on hosts is described by a negative binomial distribution. The fraction of hosts escaping infection f(P) is then given by the zero term of the negative binomial, according to which,

$$\mathrm{f}(P) = \left[1 + \left(\frac{a}{k}\right)P\right]^{-k}. \qquad (5)$$

Here f(P) is the probability of escaping infection, P is the density of parasitoids, a is the proportion of the host area searched by the parasitoid, and k is the inverse of the squared coefficient of variation of the distribution of attacks. The justification for this assumption is that hosts are assumed to be distributed among discrete patches, while the parasitoids are assumed to first disperse among patches, and then to search within patches for hosts to attack (May, 1978). Under these assumptions, if the distribution of attacks within each patch follows a Poisson distribution, then the distribution of attacks over all patches will have a variance that is greater than its mean (May, 1978). One such over-dispersed distribution is the negative binomial, which may provide a useful approximation to the overall distribution of attacks. Note that this model does not describe the distributions of host and parasitoid explicitly, nor does it allow for details of the behaviour of host or parasitoid. For these reasons, it is generally assumed to provide only a phenomenological description of the small-scale interactions between host and parasitoid (May, 1978; Hassell, 1982). It is hoped that the model will provide at least an approximate description of the small-scale interactions between the gypsy moth and its virus. The corresponding linear model with no clumping is the familiar random parasitoid model of Nicholson and Bailey (1935) where the proportion of hosts surviving is given by the zero term of the Poisson distribution:

$$\mathrm{f}(P) = \mathrm{e}^{-aP}. \qquad (6)$$

This latter model embodies an assumption analogous to the mass action assumption, in that the number of encounters with hosts (the number of attacks) N_e by P parasitoids is in direct proportion to density of hosts (N) and parasitoids ($N_e = a\ N\ P$).

The constant a has been defined as the 'area of discovery' or 'search efficiency'; a species-specific constant representing the mean proportion of the total area searched by a parasitoid in its lifetime (Nicholson, 1933; Nicholson & Bailey, 1935; Hassell, 1978, 1982). In practice, it is usually estimated by fitting models 5, 6, or a related model, to the observed fraction of hosts escaping parasitism. A substantial amount of literature has shown that a is not constant, but varies with both host and parasitoid density (see reviews by Hassell, 1978). These findings are analogous to the demonstration of non-linearity of transmission in host–pathogen models (Dwyer & Elkinton, 1993; D'Amico *et al.*, 1996; Knell *et al.*, 1998).

In short, although a full model of a baculovirus epizootic with small-scale spatial structure does not currently exist, it is possible to approximate the short-term dynamics of the virus using the negative-binomial model of parasitoid attacks. This approach is similar to that of Briggs and Godfray (1996), who replaced the νSP term in equation 1 above by the term $k \ln[1 + (\nu P/k)]S$ in an effort to produce a continuous-time approximation to the negative-binomial model.

An additional key feature of the negative-binomial model is its similarity to a previous model that incorporated host heterogeneity in susceptibility (Dwyer *et al.*, 1997). To see this, equations 2–4 were simplified to match the conditions of previous experiments. There is no change in the density of the virus after the start of the experiment, so $\mathrm{d}P/\mathrm{d}t = 0$ in equation 3, which allows equations 2–4 to be solved for the fraction of hosts F(P_0) that become infected by the end of the experiment as a function of initial virus density P_0, which is

$$\mathrm{F}(P_0) = \mathrm{e}^{(-\nu P_0 t).} \qquad (7)$$

Note that, if $\nu \times t = a$, then equation 7 is identical to equation 6, with the proviso that the units on a, in this case, depend on whether P_0 is defined to be the total number of cadavers, or the density of cadavers. Most epidemiological models assume that transmission depends on density, but for these experiments, in which the area is fixed, P_0 can alternatively be defined to be the total number of cadavers. A similar simplification and redefinition of an epidemic model that allows for heterogeneity in susceptibility gives the same model as the negative-binomial host–parasitoid model.

For the purposes of describing these experiments, then, the negative-binomial host–parasitoid model and the insect–pathogen model with heterogeneity in susceptibility are identical. Because modelling host heterogeneity in susceptibility models is simpler than allowing for small-scale spatial structure, the heterogeneity in susceptibility model has two advantages over the negative-binomial model. First, it is part of a full epidemic model, and second it can be connected to the biology more directly (Dwyer *et al.*, 1997). These advantages, however, do not mean that host heterogeneity in susceptibility is the complete explanation for the non-linear transmission of the gypsy moth virus. Indeed, the fact that the two models make identical predictions in our experiments suggests that a truly mechanistic model of this system is likely to be more complicated than either model. More practically, the identical predictions at the scale of leaves suggests that distinguishing the relative importance of these two mechanisms requires careful experimentation.

In this paper, it is tested whether or not spatial clumping affects transmission of the gypsy moth virus by determining whether experimental manipulations of pathogen clumping lead to different values of the parameter k in equation 5. The approach is to use small-scale field experiments similar to those that have been used to test models of disease transmission in insects (Dwyer, 1991; 1992; Dwyer & Elkinton, 1993; Thomas *et al*., 1995; D'Amico *et al*., 1996; Knell *et al*., 1998). In this case, the experiments involve gypsy moth larvae confined in mesh bags on foliage of red oak (*Quercus rubra*) or black oak (*Quercus velutina*) trees, contaminated with larvae killed by the gypsy moth nucleopolyhedrovirus.

To fit the model to this system the variables are redefined as follows. P is a lethal dose of pathogens; previous research suggests that each cadaver contains 10–20 lethal doses (Shapiro *et al*., 1986; J. D. Podgwaite, unpubl. data). For the parameter a two approaches are taken; first the data are fitted on survival to equation 2, second returning to the original definition of Nicholson and Bailey (1935) as the proportion of the host universe traversed by parasitoids. In this case, it is defined as the proportion of host foliage consumed by gypsy moths in the experiments, a quantity that can be measured directly.

Experimental methods: transmission experiments

In this experiment similar methods are used to those of Dwyer (1991), which are described here briefly (also see D'Amico & Elkinton, 1995; D'Amico *et al*., 1996). The basic idea behind these experiments is to confine uninfected larvae on oak branches that have been experimentally contaminated with virus-killed cadavers. The fraction of the larvae that become infected can then be used to quantify the horizontal transmission rate of the virus, which is essentially the probability of infection per unit time and per virus-infected cadaver. In all of the experiments carried out here, all of the healthy larvae are initially reared in the laboratory on an artificial diet (Bell *et al*., 1981) until the third instar, to ensure that they are uninfected. These uninfected larvae are then placed on branches that contain virus-contaminated foliage, and allowed to feed for a week. At the end of the week, the bags are removed to the laboratory, and each larva is reared in an individual cup of artificial diet until death or pupation.

In previous experiments, the source of virus used on the foliage was larvae reared on virus-contaminated diet from hatch until just before death, at which time they were placed on the experimental branches. This protocol was intended to give a natural spatial distribution of virus. In the experiments reported here, however, the aim was to manipulate the spatial distribution of the virus. Accordingly, in the first version of the experiment, in July 1993, at Otis Air National Guard Base, Massachusetts, virus-killed cadavers were used on leaves in clumped and uniform distributions. Specifically, 20 cadavers per branch in each treatment, with exactly 40 leaves on each branch. The uniform treatment had one cadaver on every other leaf (counting up from the base of the enclosed branch), while the clumped treatment had ten cadavers on one leaf and ten on another. Twenty-five healthy test larvae were then confined on each branch for 1 week, removed, and reared as previously described. A Wilcoxon signed-rank test was used to compared mean mortality in these two treatments.

Given that an effect of clumping was observed in the first experiment, in the second version of the experiment, an attempt was made to quantify the effect of clumping on transmission. To do this, the total density of cadavers per bag was varied, and the spatial clumping parameter k was used as a measure of the effect of clumping. This experiment was carried out in August 1997 on red oaks at the North-eastern Forest Experiment Station laboratory in Ansonia, Connecticut, U.S.A. Infectious cadaver densities of 0, 5, 10, 20, 40, 80, and 160 cadavers were used per 40 leaves. A random number table was used to choose leaves, and to place cadavers on leaves in either random or clumped distributions. In the random treatment, leaves were chosen randomly with replacement from the 40 leaves in the bag until each cadaver had been assigned a leaf. For all the clumped treatment densities except the five-cadaver density, five leaves were chosen at random, and cadavers were divided evenly among these leaves. For the clumped five-cadaver density, each of the five cadavers was placed on a randomly chosen leaf. Healthy test larvae were then confined on each branch for 1 week, removed, and reared as above. SAS NLIN (SAS Institute, 1988) was then used with the derivative-free option to fit equation 5 to the proportion surviving the experiment. To quantify the effects of clumping, this equation was fitted separately to each of the two spatial clumping treatments, clumped and random. Also, to test the effects of clumping, equation 5 was re-fitted using a point estimate of the area-searched parameter a that was estimated from an independent feeding experiment, to derive additional estimates of the parameter k for each spatial clumping treatment. In statistical tests for an effect of clumping, equation 5 was used as the null hypothesis, that there is no effect of clumping.

In the final version of this experiment, in May of 2000, an attempt was made to eliminate the effects of the clumping of virus in cadavers. This was carried out by using a purified virus suspension rather than infectious cadavers, so that the virus could be applied to test branches using an electronic pipette in units of single lethal doses, rather than the multiple infectious doses found in entire cadavers. The branch-wide doses that were used were 100, 200, 400, 800, and 1600 single lethal doses (an LD_{90} dose, or $\approx 1 \times 10^6$ occlusion bodies). Each set of doses were used in each of the spatial treatments, clumped and random. In the clumped treatment, the effects of having the virus clumped into cadavers was simulated by placing the virus onto leaves in the form of ten lethal doses that were contiguous on the foliage, so that the virus in the clumped treatment was applied in units of 0, 10, 20, 40, 80, and 160 groups of ten lethal doses each. In the random treatment, single lethal doses were placed individually and randomly on the foliage

according to a computer-generated table. The overall density of the virus in the two treatments, however, was identical. To assess the ability of each model, equation 5 or equation 6, to describe the data, a lack of fit test was carried out, which tests whether the data can reject a particular model. To do this, an approximate F statistic was calculated: $F = [(\mathrm{SSE} - \mathrm{SSPE})/(g - p)]/[\mathrm{SSPE}/n - g]$, where SSE is the model error sum of squares, SSPE is the 'pure' error sums of squares for a model that allows for a different mean response for each dose level and is thus a best-case regression model, n is the number of reps (bags), g is the number of dose levels and p is the number of parameters in the model (Neter *et al.*, 1996). This was compared with an F distribution with $g - p$ and $n - g$ degrees of freedom. A significant F indicates rejection of the model. To determine if the clumping parameter k for the model 6 was significantly different between clumped and random treatments, an approximate Z statistic was calculated: $Z = (|k_{\mathrm{cl}} - k_{\mathrm{ra}}|)/\sqrt{[(\mathrm{SE}_{k\mathrm{cl}})^2 + (\mathrm{SE}_{k\mathrm{cra}})^2]} \approx N(0,1)$.

Measuring the area consumed by a gypsy moth larva

The virus transmission experiments that have been described above were aimed at manipulation of the small-scale spatial patchiness of the virus, and thus have to do with the spatial clumping parameter k in equation 2. In contrast to k, the parameter a, which represents the fraction of the leaf area that is consumed by a host gypsy moth, can be estimated independently of virus transmission data, at least in theory. Another way of testing the adequacy of equation 2 is thus to estimate a by measuring the feeding rate of gypsy moth larvae, and then comparing this estimate to an estimate derived by fitting a to the virus transmission data.

To measure a independently of the transmission experiments here, 30 gypsy moth larvae were confined inside mesh bags on tree branches with five to eight undamaged black oak leaves for 24 h, using one insect per bag. The area eaten was then multiplied by seven to give a value appropriate for the 7-day transmission experiments described below. This experiment ran for only 24 h because the amount of damage occurring after the full 7 days makes it difficult to determine the original dimensions of a leaf. The leaves were removed from the tree and returned to the laboratory. The area of foliage missing from damaged leaves was measured by tracing the outlines of leaf damage onto the graph paper, and adding the areas of the whole and partial squares within the traced outlines.

Results

The effects of clumping in these experiments were roughly consistent across different experiments. In the first experiment (Fig. 1), in which only one virus density was used, mortality in the clumped treatment was significantly less than in the uniform treatment ($n = 17$, $P < 0.001$) even though the virus density per leaf was the same. In the second experiment, in which multiple virus densities were used, transmission was a non-linear function of virus density in both the clumped and randomly distributed treatments (Fig. 2). Note

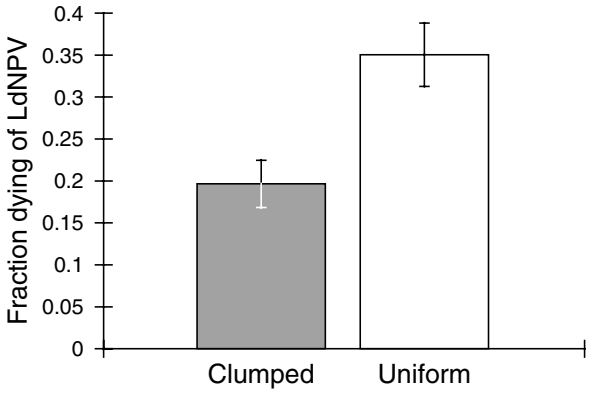

Fig. 1. Fraction of third-instar gypsy moth larvae dying from LdMNPV after 1 week confinement on foliage contaminated with first instar LdMNPV-killed larvae. LdMNPV-killed first instars were placed on individual leaves in either a uniform or clumped pattern. Error bars represent one standard error of the mean proportion.

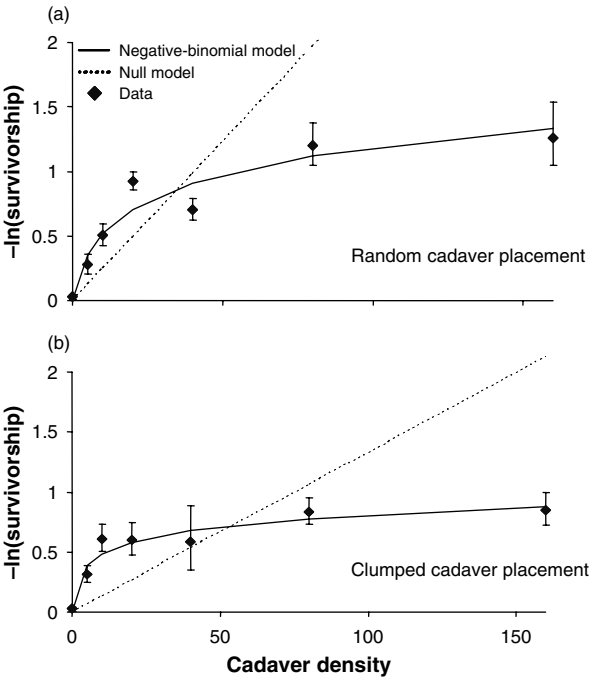

Fig. 2. The natural log transformed mean survivorship of third-instar gypsy moth larvae after 1 week confinement on foliage contaminated with first instar LdMNPV-killed larvae. LdMNPV-killed first instars were placed on individual leaves in either a random (a) or clumped (b) distribution, as determined by a computer-generated random number table. Data are represented by diamonds, and curves are the best-fit negative-binomial model (May, 1978) (solid lines) and null model (dotted lines). Error bars represent one standard error of the mean proportion.

that, to present and analyse the data, the negative natural log of the fraction infected was used, which makes it easier to see the difference between the linear and non-linear transmission models, while preserving the property that mortality increases along the vertical axis. For the clumped treatment the lack-of-fit of the non-linear model was $F_{5,26} = 2.21$, $P = 0.08$ compared with $F_{6,26} = 11.3$, $P < 0.001$ for the linear model. For the random treatment the lack-of-fit of the non-linear model was $F_{5,24} = 1.81$, $P = 0.15$ compared with $F_{6,24} = 16.1$, $P < 0.001$ for the linear model. The data from both the clumped and random treatments thus reject the linear model, but not the non-linear model. The best-fit heterogeneity parameter k for the clumped cadavers was 0.144 ± 0.048 SE compared with 0.318 ± 0.068 SE for the random cadavers, and these were significantly different ($Z = 2.09$, $P = 0.037$). As indicated above, the significantly higher values of k in the randomly placed cadavers indicate lower degrees of clumping or heterogeneity in the fit of model 2 to the mortality data from the random compared with the clumped treatment.

In the third experiment, when the doses were droplets of LdNPV rather than cadavers, transmission in both treatments was more nearly linear (Fig. 3), but the data reject the linear model only in the random treatment. For the clumped treatment, the lack-of-fit of the non-linear model was $F_{4,42} = 0.79$, $P = 0.53$ compared with $F_{5,42} = 1.77$, $P = 0.14$ for the linear model. For the random treatment the lack-of-fit of the non-linear model was $F_{4,42} = 0.53$, $P = 0.71$ compared with $F_{5,42} = 3.10$, $P = 0.017$ for the linear model. Nevertheless, the strength of the clumping effect was much weaker, in that the estimated clumping parameters were $k = 1.58$ for the clumped treatment and $k = 1.50$ for the random treatment. The values of these parameters were not significantly different from each other ($Z = 0.81$, $P = 0.93$), but both were less clumped (higher k) than the corresponding values in the second experiment with cadavers (clumped treatment: $Z = 1.57$, $P = 0.12$; random treatment: $Z = 2.91$, $P = 0.003$).

To test the transmission of a virus at a mechanistic level, the area-searched parameter a was also measured, as the area of foliage eaten per day by a third-instar larva over 7 days, which was 0.82 ± 0.12 cm^2. The corresponding value of a for third instars, that is the estimated proportion of foliage consumed over 7 days was $a = 0.0175 \pm 0.0026$ for the second experiment. The corresponding values of a as calculated by fitting equation 5 to the data from the second transmission experiment, which used cadavers, were of $a = 0.013 \pm 0.004$ SE for the random treatment and $a = 0.040 \pm 0.05$ SE for the clumped treatment (Table 1). The similarity of these best-fit values of a to the experimental estimate of a suggests that the model may incorporate the correct mechanism of transmission in the gypsy moth. Nevertheless, the values for the third experiment, which used droplets of virus suspension instead of cadavers, were quite different; for that experiment, $a = 0.0016 \pm 0.0026$ SE for the random treatment and $a = 0.0031 \pm 0.0031$ SE for the clumped treatment (Table 1).

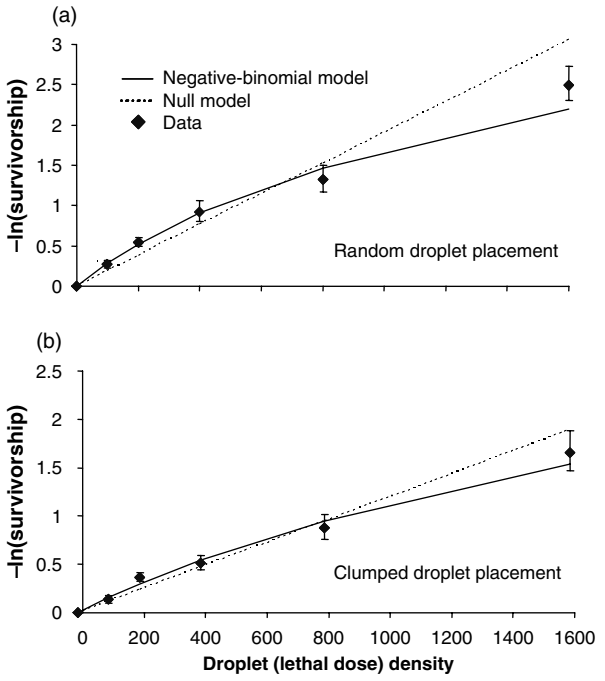

Fig. 3. The natural log-transformed mean survivorship of third-instar gypsy moth larvae after 1 week confinement on foliage contaminated with droplets of LdMNPV suspension in distilled water. Droplets were placed on foliage in either a random (a) or clumped (b) distribution, as determined by a computer-generated random number table. Data are represented by diamonds, and curves are the best-fit negative-binomial model (May, 1978) (solid lines) and null model (dotted lines). Error bars represent one standard error of the mean proportion.

Discussion

In general, the results show a strong effect of the clumping of cadavers on disease transmission in gypsy moth larvae. As in Dwyer (1991), the first experiment showed simply that clumping reduces transmission rates. The second and third transmission experiments, however, showed that clumping can have a strong effect on the degree of non-linearity in the transmission of this virus. In the across-density experiment using virus-killed cadavers, both of the experimental treatments (clumped cadavers vs. randomly placed cadavers) showed sublinear transmission, although there was a significant difference between k-values calculated for the treatments. The lethal doses of pathogens remained highly clumped within the cadavers (10–20 per cadaver) in *both* treatments in these experiments, and this could explain the remaining non-linearity evident in the random treatment. Indeed, in the experiment in which droplets of virus were used, which spreads evenly and thinly on foliage, the non-linearity was eliminated altogether. Spatial clumping may therefore provide at least a partial explanation for non-linear transmission in this host–pathogen

Table 1. Parameter estimates (± SE) for a and k of the negative binomial for experiment (2) with cadavers and experiment (3) with LdMNPV droplets comparing clumped and random treatments and two methods of parameter estimation. In 'Experiment' (see third column), a is estimated from the feeding experiment and k is estimated by fitting model with SAS NLIN; in 'Best fit' both a and k are estimated simultaneously with SAS NLIN. P is the probability that ks differ between clumped and random treatments for a given experiment and method of fitting.

Experiment	Treatment	Estimation of a	a	k	P
Cadavers (2)	Clumped	Experiment	0.0175 ± 0.0026	0.189 ± 0.024	0.01
Cadavers (2)	Random	Experiment	0.0175 ± 0.0026	0.276 ± 0.026	
Cadavers (2)	Clumped	Best fit	0.040 ± 0.048	0.144 ± 0.048	0.04
Cadavers (2)	Random	Best fit	0.0130 ± 0.005	0.318 ± 0.068	
Droplets (3)	Clumped	Experiment	0.0175 ± 0.0026	0.174 ± 0.021	0.001
Droplets (3)	Random	Experiment	0.0175 ± 0.0026	0.295 ± 0.032	
Droplets (3)	Clumped	Best fit	0.0016 ± 0.00026	1.58 ± 0.802	0.94
Droplets (3)	Random	Best fit	0.0031 ± 0.0031	1.50 ± 0.515	

interaction. Furthermore, the similarity of the value of the area-searched parameter a from our feeding experiment to the value estimated from the cadaver transmission experiment suggests that a model that incorporates feeding rate and spatial clumping is a reasonable approximation to the dynamics of transmission in this virus.

Nevertheless, the difference in the degree of non-linearity between the cadaver experiment and the droplet experiment suggests that there are mechanisms operating in this system that are not considered by the spatial-clumping model. Specifically, it is suspected that some aspect of the behaviour of gypsy moth larvae besides their feeding rate affects transmission. First, though, it can be noted that overall levels of mortality were considerably higher in the droplet experiment than in the cadaver experiment, even though the total amount of virus in the two experiments was roughly the same, and even though the total leaf area covered by virus in the two experiments was also roughly the same. Given these observations, it is suspected that virus in the form of cadavers is qualitatively different than virus that has been purified. For example, it may be the case that larvae avoid cadavers but are not repelled by purified virus. In fact, in laboratory choice tests it has been shown that larvae cannot distinguish leaf discs contaminated with purified virus from leaf discs that are uncontaminated (V. D'Amico, unpubl. data). This or other details of the behaviour of larvae may interact with spatial structure to determine the transmission of this virus. Moreover, in previous work, it was shown that non-linearity in transmission may alternatively be explained by heterogeneity in susceptibility (Dwyer *et al*., 1997). At the time, the belief was that this heterogeneity in susceptibility was due to heterogeneity in the dose required to infect a larvae; additional work, however, has instead suggested that heterogeneity in feeding rate is more important (G. Dwyer, unpubl. data). The ultimate explanation for non-linear transmission in the gypsy moth–virus interaction is therefore likely to involve some combination of spatial clumping and heterogeneity in behaviours such as feeding rate or the ability to avoid virus-killed cadavers.

A deeper understanding of how spatial structure affects the transmission of baculoviruses is necessary before the effects of spatial clumping on the dynamics of the gypsy moth–virus interaction can fully considered. For the time being it is noted that the levels of non-linearity in transmission observed in these experiments, as measured by the clumping parameter k, are sufficient to turn stable cycles into stable equilibria in most models of insect–pathogen interactions (Briggs & Godfray, 1996, Dwyer *et al*., 2000). The non-linearity in transmission seen in these experiments is thus sufficient to have a strong effect on the dynamics of host–pathogen interactions; more generally, this work shows that spatial structure and behaviour can play a key role in such systems.

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References

Bell, R.A., Owens, C.D., Shapiro, M. & Tardiff, J.R. (1981) Mass rearing and virus production. *The Gypsy Moth: Research Toward Integrated Pest Management* (ed. by C. C. Doane and M. L. McManus), pp. 599–655. USDA Technical Bulletin 1584, USDA, Washington, DC.

Bolker, B.M. & Pacala, S.W. (1999) Spatial moment equations for plant competition: understanding spatial strategies and the advantages of short dispersal. *American Naturalist*, **153**, 575–602.

Bonsall, M. (2004) The impact of diseases and pathogens on insect population dynamics. *Physiological Entomology*, **29**, 223–236.

Boots, M. (2004) Modelling insect diseases as functional predators. *Physiological Entomology*, **29**, 237–239.

Briggs, C.J. & Godfray, H.C.J. (1996) The dynamics of insect–pathogen interactions in seasonal environments. *Theoretical Population Biology*, **50**, 149–177.

Cory, J.S., Hails, R.S. & Sait, S.M. (1997) Baculovirus ecology. *The Baculoviruses* (ed. by L. K. Miller), pp. 301–339. Plenum, New York.

D'Amico, V. & Elkinton, J.S. (1995) Rainfall effects on the transmission of gypsy moth nuclear polyhedrosis virus. *Environmental Entomology*, **24**, 144–1149.

D'Amico, V., Elkinton, J.S., Dwyer, G., Burand, J.P. & Buonaccorsi, J.P. (1996) Transmission of gypsy moth (Lepidoptera: Lymantriidae) virus is not a simple mass-action process. *Ecology*, **77**, 201–206.

D'Amico, V., Elkinton, J.S., Dwyer, G., Willis, R.B. & Montgomery, M.E. (1998) Foliage damage does not affect within-season transmission of an insect virus. *Ecology*, **79**, 1104–1110.

Dwyer, G. (1991) The roles of density, stage, and patchiness in the transmission of an insect virus. *Ecology*, **72**, 559–574.

Dwyer, G. (1992) On the spatial spread of insect pathogens: theory and experiment. *Ecology*, **73**, 479–494.

Dwyer, G. & Elkinton, J.S. (1993) Using simple models to predict virus epizootics in gypsy moth populations. *Journal of Animal Ecology*, **62**, 1–11.

Dwyer, G., Elkinton, J.S. & Buonaccorsi, J. (1997) Host heterogeneity in susceptibility and disease dynamics: tests of a mathematical model. *American Naturalist*, **150**, 685–707.

Dwyer, G., Dushoff, J., Elkinton, J.S. & Levin, S.A. (2000) Pathogen-driven outbreaks in forest defoliators revisited: building models from experimental data. *American Naturalist*, **156**, 105–120.

Hails, R.S., Rees, M., Kohn, D.D. & Crawley, M.J. (1997) Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. *Proceedings of the Royal Society of London B*, **264**, 1–7.

Hassell, M.P. (1978) *The Dynamics of Arthropod Predator–Prey Systems*. Princeton University Press, Princeton, New Jersey.

Hassell, M.P. (1982) Patterns of parasitism by insect parasitoids in patchy environments. *Ecological Entomology*, **7**, 365–374.

Knell, R.J., Begon, M. & Thompson, D.J. (1998) Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: a test of the mass action assumption with an insect pathogen. *Proceedings of the Royal Society of London B*, **263**, 75–81.

Lewis, M. & Pacala, S. (2001) Modeling and analysis of stochastic invasion processes. *Journal of Mathematical Biology*, **41**, 387–429.

May, R.M. (1978) Host–parasitoid systems in patchy environments: a phenomenological model. *Journal of Animal Ecology*, **47**, 833–840.

McCallum, H., Barlow, N. & Hone, J. (2001) How should pathogen transmission be modelled? *Trends in Ecology and Evolution*, **16**, 295–300.

Murray, K. & Elkinton, J. (1989) Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). *Journal of Invertebrate Pathology*, **53**, 324–334.

Murray, K. & Elkinton, J. (1990) Transmission of nuclear polyhedrosis virus to gypsy moth (Lepidoptera: Lymantriidae) eggs via contaminated substrates. *Environmental Entomology*, **19**, 662–665.

Neter, J., Kutner, M., Nachtsheim, C. & Wasserman, W. (1996) *Applied Linear Statistical Models*, 4th edn. McGraw-Hill/Irwin, New York.

Nicholson, A.J. (1933) The balance of animal populations. *Journal of Animal Ecology*, **2**, 131–178.

Nicholson, A.J. & Bailey, V.A. (1935) The balance of animal populations. Part I. *Proceedings of the Zoological Society of London*, **3**, 551–598.

SAS Institute (1988) *SAS/STAT User's Guide, Release 6.03 Edition*. SAS Institute, Cary, North Carolina.

Shapiro, M., Robertson, J.L. & Bell, R.A. (1986) Quantitative and qualitative differences in gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus produced in different-aged larvae. *Journal of Economic Entomology*, **79**, 1174–1177.

Thomas, M.B., Wood, S.N. & Lomer, C.J. (1995) Biological control of locusts and grasshoppers using a fungal pathogen, the importance of secondary cycling. *Proceedings of the Royal Society of London B*, **259**, 265–270.

Woods, S.A. & Elkinton, J.S. (1987) Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar* L.) populations. *Journal of Invertebrate Pathology*, **50**, 151–157.

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