A model of Nucleopolyhedrovirus (NPV) population genetics applied to co-occlusion and the spread of the few polyhedra (FP) phenotype

H. C. J. GODFRAY, D. R. O'REILLY AND C. J. BRIGGS*

Department of Biology and NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, UK

SUMMARY

A strategic model of Nucleopolyhedrovirus (NPV) genetics is developed and applied to co-occlusion and the dynamics of the few polyhedra (FP) phenotype. Co-occlusion is the incorporation of wild-type and mutant viruses in the same occlusion body as a strategy to deliver genetically modified viruses as an insecticide in a way that minimizes their persistence in the environment. The model predicts that the persistence of the modified virus depends most critically on the number of viruses that infect an occlusion body-producing nucleus, the number of occlusion bodies typically ingested by an insect, and the number of viruses that cross the gut wall. FP viruses occur at high frequency in cell culture where they have a replication advantage over wild-type virus. They cannot, however, produce viable occlusion bodies, but it is shown that under certain circumstances they can persist in natural populations as parasites of wild-type viruses. FP viruses have not as yet been identified in natural NPV populations, and possible reasons for this are discussed. We suggest that the models developed here can also help in studying potential recombination between engineered and wild-type viruses in the environment.

1. INTRODUCTION

Baculoviruses are double-stranded circular DNA viruses that are major pathogens of invertebrates, particularly insects (Granados & Federici 1986; Francki *et al.* 1991). A feature of these viruses is the formation of proteinaceous occlusion bodies around virus particles in infected cells. There are two genera, *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) that are distinguished by the number of virus particles per occlusion body. NPVs form large polyhedral occlusion bodies each containing multiple virus particles whereas GVs form smaller, oval-shaped occlusion bodies each containing only a single particle.

NPVs attack many important pests and some have been developed as biopesticides in agriculture and forestry. In recent years, the possibility of improving NPV efficiency by genetic manipulation has received considerable attention (Wood 1991; Wood & Granados 1991; Wood & Hughes 1995; Bonning & Hammock 1996). The possible release of viruses containing transgenes raises many safety issues. For example, what is the risk that manipulated viruses will move into natural environments and detrimentally affect non-target organisms, and what is the possibility that the transgene will move by recombination into

other viruses or even other organisms (Crook & Winstanley 1995; Godfray 1995; Huber 1995; Whitten 1995)?

Addressing these questions clearly requires extensive experimental studies, but also a conceptual framework to interpret the results. However, NPVs have unusual population genetics and standard models cannot be applied directly. The main aim of this paper is to develop a general conceptual framework for the study of gene frequency changes in NPVs. We do this by modelling two specific problems: the co-occlusion of wild-type and disabled viruses in biopesticide sprays, and the evolution of the 'few polyhedra' (FP) phenotype. We also argue that models of the type developed here can be used to obtain information on aspects of NPV biology that are important in assessing the risk of genetic manipulation, for example the likelihood of recombination between different strains.

(a) NPV biology

Insects are infected by eating food contaminated by occlusion bodies which are typically 1–5 μm in diameter and contain in the order of 100 viral genomes. A distinction is made between two categories of NPVs, singly-embedded NPVs (SNPVs), where each nucleocapsid is embedded independently in the occlusion body matrix, and multiply embedded NPVs (MNPVs), where nucleocapsids are embedded in

* Present address: Department of Ecology, Evolution & Marine Biology, University of California, Santa Barbara, CA 93106, USA.

groups within the same outer membrane. In baculovirus biology, the term nucleocapsid is used for a single virus genome surrounded by structural proteins, while a virion or virus comprises one or more nucleocapsids surrounded by a lipid envelope.

On ingestion, the protein matrix of the occlusion body breaks down in the high pH of the insect midgut and the liberated virus infects the cells of the midgut epithelium. Progeny virus is initially released from infected cells as budded virus, single virus particles surrounded by a lipid envelope. This budded virus goes on to infect other cells in the insect. Later in infection, progeny virus is retained within the nucleus of the infected cell, where it becomes incorporated within occlusion bodies. Eventually, a large proportion of the insect's cells are infected and its whole body liquefies, releasing occlusion bodies into the environment. The occlusion body serves to stabilize the virus in the environment and allows the pathogen to persist for long periods of time in protected reservoirs such as the soil.

(b) *Co-occlusion and the FP phenotype*

The strategy of co-occlusion was originally proposed to counter the possible risks of releasing manipulated viruses into the environment (Miller 1988; Hamblin *et al.* 1990; Wood *et al.* 1994; Wood & Hughes 1995). A foreign gene is engineered into the virus so that it replaces the polyhedrin gene. Polyhedrin is the major component of the occlusion body so the recombinant virus is occlusion-negative and would be inactivated very rapidly in the field. However, wild-type (occlusion-positive) and recombinant virus can be grown together in cell culture so that recombinant genomes are co-occluded into occlusion bodies formed by wild-type virus. An insect infected by such an occlusion body should express the desired foreign gene, but should produce relatively few occlusion bodies containing the manipulated virus. Over a number of generations, the manipulated genotype should be lost in competition with the wild-type (the position of the inserted gene makes it unlikely that recombination will generate a virus containing both the polyhedrin and foreign genes). This system represents a simple example of two alleles of a gene segregating in the viral population. Here, we use it as a starting point for the development of a model of NPV population genetics, and explore what factors determine the rate at which occlusion-negative virus is lost from the population after application.

NPVs can be maintained in cell culture where they are transmitted between cells in the budded virus form. After passaging for a few generations, mutants with characteristic features are very frequently observed (Potter *et al.* 1976, 1978; Fraser & Hink 1982; Burand & Summers 1982; Burand *et al.* 1983; Fraser *et al.* 1983; Miller 1986; Blissard & Rohrmann 1990). Plaques formed by these mutants contain very few occlusion bodies in each infected cell and the mutants are therefore known as few polyhedra (FP) mutants. Electron microscopy has shown that those occlusion bodies that are formed contain few or no nucleocapsids.

However, FP mutants give increased yields of budded virus, so that they enjoy a selective advantage over wild-type virus in cell culture. The avoidance of FP mutants is a major concern in the use of baculoviruses as a large-scale gene expression system (Tramper & Vlak 1986). Because of the simultaneous inheritance of multiple genome copies, it is possible for FP mutants to persist in wild populations of NPVs, in effect acting as genetic parasites. The limited available evidence suggests this does not occur (Potter *et al.* 1978; Fraser & Hink 1982), and we use the model to explore the conditions under which FP mutants may or may not persist. The biology of FP mutants is related to that of defective forms of other types of virus that are unable to replicate without the presence of fully functional viruses providing *trans*-acting factors (Huang & Baltimore 1970; Holland 1990).

Throughout what follows we shall assume the virus has discrete generations and that the host population is constant in size, and made up of identical individuals. It is straightforward to relax these assumptions at the cost of a more complex model.

2. CO-OCCLUSION

Consider two viral types: P^+ the normal wild-type and P^- a recombinant virus lacking the polyhedrin gene. Let x represent the fraction of P^+ virus in an occlusion body and $p_t(x)$ the probability distribution of x across all occlusion bodies in generation t . We seek to derive the probability distribution in the next generation and to use the recurrence equation to study the fate of the P^- genotype.

(a) *A simple model*

We begin with a very simple model of baculovirus ecology and genetics, but then relax the major assumptions. Assume first that (i) all hosts are infected by a single occlusion body and that (ii) sufficient nucleocapsids from the occlusion body infect the host that the frequency of wild-type viral genomes in the insect and the occlusion body are the same. If we call the frequency of P^+ viruses in infected insects z , and define the probability distribution of z across hosts in generation t as $r_t(z)$, then at this stage we are making the assumption that $r_t(z) = p_t(x)$.

Given that an insect is suffering a mixed infection of P^+ and P^- viral types, we need to know the genetic composition of the occlusion bodies that result. Occlusion bodies are assembled in individual cells and thus a critical factor is the probability of a cell being coinfecting by different viral genotypes. Let $g(i)$ be the fraction of cells that are infected by $i = \{1, 2 \dots n\}$ nucleocapsids — the multiplicity of cell infection. We assume that cell infection by different nucleocapsids is statistically independent. (This may not be so if cells are infected by viruses produced by adjacent cells, but our model will still describe the dynamics of infection if we interpret i as the number of independent infections by groups of genetically identical nucleocapsids.)

A second important factor is the relative reproductive performance of the two viral genotypes in

producing budded virus and spreading through the host. Conceivably, genetic engineering could result in a virus with either enhanced or reduced replication ability. Here we concentrate on the normative case where the P^+ and P^- viral types have identical replicative potentials and only differ in that no occlusion bodies are produced in cells infected solely by polyhedrin minus (P^-) viruses. Thus if a cell is infected by k type P^+ viruses and l type P^- viruses, it will produce occlusion bodies with $x = k/(k+l)$ as long as $k \geq 1$.

Before continuing, a technical point has to be made. The possible frequencies of the two viral genotypes in an occlusion body, x , are determined by the maximum number of nucleocapsids infecting a cell. For example, if $n = 4$ then x belongs to the set $\phi(n) = \phi(4) = \{1/4, 1/3, 1/2, 2/3, 3/4, 1\}$. $\phi(n)$ is the set of all rational numbers k/i , $i = \{1, 2 \dots n\}$, $k = \{1, \dots i\}$. (Note, zero is not included as at least one P^+ virus must be present for occlusion body formation.) The probability distributions $p_t(x)$ and $r_t(z)$ are thus not continuous and summations involving these distributions are taken over this set. Further, if the joint probability distribution of k and i is known, $\phi_t(k, l)$ where $k+l = i$, then the probability distribution of the elements of $\phi(n)$ is fully specified by the function we define as $\Omega[\phi_t(k, l)]$.

We first write down an expression for, $\phi_t(k, l)$, the probability distribution of occlusion body-producing cells (across all insects in the population) infected by $k+l$ viruses of which k are P^+ . Because at least one P^+ must be present for occlusion body formation, $k \geq 1$.

$$\phi_t(k, l) = \sum_{z \in \phi(n)} r_t(z) \left[\frac{g(k+l) \binom{k+l}{k} z^k (1-z)^l}{1 - \sum_{i=1}^n g(i) (1-z)^i} \right]_{k+l = \{1, 2 \dots n\}, k \geq 1. \quad (1)$$

The equation is derived as follows. In an insect infected by a fraction z of the P^+ virus, the number of occlusion body-producing cells that contain exactly k P^+ and l P^- viruses is proportional to the product of the probability that a cell contains $k+l$ viruses in total (given by $g(k+l)$) and the binomial probability of k out of $k+l$ successes with parameter z . To convert this quantity to a frequency we have to allow for the cells that are infected only by P^- viruses and hence produce no occlusion bodies. This normalization is responsible for the denominator in equation (1). Finally, we are interested in the probability distribution across all occlusion body-producing cells in the population and so we sum across all insects weighted by the probability that they were infected by consuming an occlusion body containing a fraction z of the P^+ virus.

Given the distribution, $\phi_t(k, l)$, we can calculate the frequencies of the two virus types in the occlusion bodies of the next generation. Occlusion bodies contain a large number of nucleocapsids (typically around 100) which allows us to assume that the genotype frequencies in the occlusion body and its mother cell are effectively identical.

The model described above is fully specified apart from the function $g(i)$. There is very little evidence available that gives an indication of how many

Figure 1. (a) The number of generations taken for the frequency of an occlusion-minus genotype to drop from 50% to 1% after an initial application of co-occluded particles. The distribution of nucleocapsids per occlusion body-producing cell is described by the parameter α of the truncated geometric distribution (see text) with larger numbers indicating more cells infected by single nucleocapsids. In this figure we assume all infections result from the ingestion of a single occlusion body with many of the nucleocapsids it contains crossing the gut wall. (b) As (a) but now we assume infection results from the ingestion of many occlusion bodies.

nucleocapsids infect a cell. We have chosen to explore the model using a truncated geometric distribution with a maximum number of viruses per cell of $n = 4$. To some extent this is an arbitrary choice but we have obtained very similar results with other monotonically decreasing probability distributions. This truncated geometric distribution is described by a single parameter, α , which we vary from zero to one. When $\alpha = 0$, there is an equal likelihood of cells being infected by 1, 2, 3 or 4 virions. As α increases, a greater fraction of cells are infected by a single nucleocapsid until when $\alpha = 1$ no cell is multiply infected.

(b) Results

Suppose we introduce into the environment a suspension of occlusion bodies each containing 50%

wild-type (P^+) and 50% occlusion-negative (P^-) viruses. The predictions of the model are shown in figure 1*a*. The P^- genotype is outcompeted by the P^+ genotype because it suffers the selective disadvantage of being unable to synthesize occlusion bodies. The extent of this competitive disadvantage, and hence the speed with which P^- is lost from the environment, depends critically on the probability that insect cells are infected by more than one nucleocapsid. If all cells are singly infected ($\alpha = 1$) then P^- is lost after one generation while if there is an equal probability of infection by 1, 2, 3 or 4 viruses ($\alpha = 0$), it takes 12 generations for the P^- virus to drop to a frequency of 1%.

(c) Infection caused by multiple occlusion bodies

We have assumed so far that insects are infected by ingesting a single occlusion body. We now turn to the opposite extreme and suppose the insect ingests a very large number of occlusion bodies. The frequency of P^+ in the insect gut is now simply the average across all occlusion bodies. Further, we assume a sufficient number cross the gut wall that the frequency of P^+ in the insect itself (z_t^*) is the same average. Thus z^* is constant across all insects and given by

$$z_t^* = \sum_{x \in \phi(n)} x p_t(x). \quad (2)$$

Figure 1*b* shows the rate of loss of the P^- genotype for different distributions of viruses per cell. Compared with figure 1*a*, P^- is lost more slowly. When infection occurs through ingestion of a single occlusion body, the descendants of a pure P^+ infection can never come into contact with a P^- virus. Over a number of generations, the frequency of pure P^+ lineages increases and the P^- virus declines in frequency. In the case of multiple infections, the elimination of P^- viruses occurs more slowly because pure P^+ lineages are not formed and all wild-type viruses are available in mixed infections to produce occlusion bodies containing both viral types.

(d) Few nucleocapsids cross the gut wall

The second major assumption in the simple model is that a large number of nucleocapsids cross from the gut into the insect. Here we relax this assumption and assume that only a limited number infect the insect. We suppose that the insect consumes a large number of occlusion bodies, as in the last section, so that the frequency of viral genotypes in the gut is equal to the population mean across occlusion bodies. The distribution of genotype frequencies across insects ($r_t(z)$) is then determined by random sampling of the nucleocapsids in the gut. Given $r_t(z)$, the frequency of viral genotypes in the next generation of occlusion bodies can be calculated using equation (1).

Figure 2 shows how limited infection influences the loss of the P^- genotype. We consider the six cases of when the gut wall is crossed by (i) a very large number of viruses (i.e. the model of the previous section); exactly (ii) 4, (iii) 3 or (iv) 2 viruses; and 2 or 1 viruses

Figure 2. The influence of a limited number of nucleocapsids crossing the gut wall. The six clusters of histograms represent decreasing numbers of nucleocapsids crossing the gut wall: (i) the limit as many cross the gut wall; (ii) 4; (iii) 3; (iv) 2; (v) 1 or 2 in different cells with equal probability; (vi) 1 nucleocapsid with probability 0.75, 2 nucleocapsids with probability 0.25. Each histogram cluster is made up of six bars representing different distributions of nucleocapsids per occlusion body-producing cell (given by the truncated geometric distribution with parameter $\alpha = \{0, 0.2, 0.4, 0.6, 0.8, 1\}$; the same variable as on the x axis of figures 1 and 2).

in ratios (v) 1:1 and (vi) 1:3. Note that if only one nucleocapsid crosses the gut wall, co-infection is impossible and the P^- virus is lost immediately. The introduction of a bottleneck at the gut wall increases the speed at which the P^- virus is lost because it reduces the amount of genetic mixing between the two viral genotypes. However, major effects on the speed of loss are only apparent when the numbers crossing the gut wall are very small.

3. THE FP PHENOTYPE

Consider now an FP mutant that has a defect in the production of occlusion bodies but has a replication advantage during the production of budded viruses. We can study the population genetics of this mutant using a simple modification of the co-occlusion model. We assume that all infected cells produce the same number of occlusion bodies unless they contain only FP mutants, in which case they produce none. (In fact, cells infected solely by an FP mutant will produce a small number of occlusion bodies but these have very low infectivity (Fraser & Hink 1982; Slavicek *et al.* 1995.)) We assume that immediately after infection the proportion of wild-type to FP genotypes in the insect is $z:(1-z)$. However, because of their replication advantage, the frequency of FP types increases during the rounds of budded virus production so that the ratio of the two genotypes available for infecting occlusion body-producing cells is $z':(1-z')$. We seek to describe the dynamics of the FP genotype and to predict the conditions that will allow the mutant to persist in the population.

Begin by assuming that hosts are invariably infected by the nucleocapsids liberated from a single occlusion body. In these circumstances, and in the absence of recurrent mutation, the FP mutant is invariably lost from the population. To see why, suppose that in the current generation (t) a fraction a_t of hosts are infected by pure wild-type occlusion bodies. If all infected hosts produce the same number of secondary infections in the next generation, then $a_{t+1} > a_t$. All the secondary infections produced by a wild-type infected host will themselves contain only wild-type viruses, but as hosts containing both viral alleles will produce some pure wild-type occlusion bodies, some of the secondary infections they produce will also contribute to a_{t+1} . Thus a_t always increases: a replicative advantage for the FP mutant can slow its decline, but not reverse it. In these circumstances, FP can only be maintained by recurrent mutation.

We now examine the dynamics of the FP mutation when hosts are infected by (i) two occlusion bodies and (ii) many occlusion bodies. In both cases we assume

many viral nucleocapsids cross the gut wall so that the initial viral genome frequencies in the insect (z) equals the genome frequencies in the gut. We assume that the replication advantage (W) of the FP virus leads to a linear increase in the ratio of the two viral types, such that

$$\frac{(1-z')}{z'} = W \frac{(1-z)}{z}, \quad (3)$$

where z and z' are defined above. The dynamics of FP can be studied using the co-occlusion model but substituting z' for z .

The model predictions for selected values of α and W are shown in figure 3. In contrast to the previous situation, FP mutants are not always driven to extinction but can reach an equilibrium with the wild-type virus (a stable polymorphism). The persistence and equilibrium frequency of the FP genotype is enhanced by three factors: (i) greater replication advantage; (ii) a higher probability of multiple infections of occlusion body-producing cells; and (iii) infections caused by the ingestion of larger numbers of occlusion bodies. The polymorphism is maintained by the balance of the selective advantage of the FP virus at the stage of viral spread through the insect's tissue and the selective disadvantage of the FP virus during occlusion body formation. Of the three factors that favour the FP virus, the first increases the selective advantage of FP at the viral spread stage while the second two decrease the disadvantage of FP during occlusion body formation.

4. DISCUSSION

NPVs have an unusual biology in that several copies of the genome can be inherited together within the same occlusion body. Our broad aim here has been to construct a conceptual model of NPV genetics incorporating what we believe are the main features of the virus's natural history: the infection process, the movement of viruses across the gut wall, and the infection of individual cells prior to occlusion body formation. We believe this conceptual framework can be applied to a variety of questions in baculovirus biology and illustrate this by examining two issues, co-occlusion and the production of the FP phenotype. NPV molecular genetics are relatively well explored because of their importance as eukaryote gene expression vectors, and their potential importance as engineered insecticides. However, less is known about the cellular processes of disease progression and a role for this type of model is the identification of those parts of the life cycle likely to have the greatest influence on viral population genetics.

Although we have included some aspects of viral natural history, we have omitted or simplified other aspects in order to produce a relatively tractable model. For example, we have assumed that all insects are of a single species, suffering identical infections, and have ignored the effect of insect age or any genetic variability in disease susceptibility. We have also assumed homogenous within-host disease progression and have not attempted to model the spread of the

Figure 3. The equilibrium frequency of the FP virus as a function of W , its replication advantage, and the distribution of nucleocapsids across occlusion body-producing cells. The numbers on the four curves are the parameter α of the truncated geometric distribution. (a) Insects infected by exactly two occlusion bodies. (b) Insects infected by many occlusion bodies.

infection from the gut wall through the insect tissue. Although it is technically straightforward to include all these processes within the modelling framework developed here, further data are needed to make choices amongst the many more complex models that can be constructed. Finally, we have worked only in the frequency domain and have not studied population dynamics, although we have preliminary results that show that the model developed here can be embedded in population dynamic models of NPV infections of the types developed by Briggs & Godfray (1995, 1996).

(a) *Co-occlusion*

We chose the phenomenon of co-occlusion of polyhedrin-negative and wild-type genomes as a simple problem to which to apply our model of NPV genetics. While there is no doubt about the technical practicality of co-occlusion, its economic feasibility is not yet proven. A proposed practical argument in favour of co-occlusion is that it is a means of applying engineered viral insecticide in a manner that will lead to the rapid loss of the novel construct from the environment (Wood & Hughes 1995). Rapid loss is advantageous as it restricts the possibility of the infection of non-target insects and reduces the opportunity for recombination between the engineered virus and wild virus present in the field. Our model shows how the speed of loss is influenced by different features of the virus's life cycle. The extent of multiple infections of occlusion body-producing cells is the single most important parameter influencing the rate of viral loss, with the number of viruses crossing the gut wall and the number of occlusion bodies contributing to an infection also being important.

Hamblin *et al.* (1990) have studied the fate of occlusion-negative genotypes of AcNPV in serial passage through *Trichoplusia ni* larvae. By altering the dose of co-occluded virus ingested by the larvae they showed that a greater proportion of occlusion bodies containing both genotypes are produced when larger numbers of nucleocapsids infect the insect, as our model predicts. They report no replicative advantage of the occlusion-negative genotype, supporting an assumption we made. However, they did find that larvae infected by high proportions of occlusion-negative genotypes produce relatively few occlusion bodies, and found suggestive evidence that infection by virions containing one nucleocapsid may be more efficient than infection by virions containing two. We agree with their suggestion that these factors may accelerate the loss of the occlusion-negative genotype.

(b) *FP phenotype*

Although FP mutants appear with great regularity when NPVs are propagated in cell culture, there is currently no evidence that FP mutants are stably maintained in viral populations propagated *per os* infection via occlusion bodies. Given that the mutant appears to occur at high frequency, at least *in vitro*, why are wild-type viruses not subject to this form of parasitism in the field? There are at least two possible

explanations. First, the majority of studies of NPV biology have focused on a comparatively small number of strains, and studies of unpurified field isolates are relatively rare. FP variants may be segregating in wild populations but have just not been observed.

Second, the model identifies a number of important features of NPV biology that might preclude polymorphism under normal circumstances. Possibly, most infections are caused by virus liberated from single occlusion bodies, or possibly only a very small number of nucleocapsids cross the gut wall and infect the insect. In addition, polymorphism is unlikely if the replicative advantage of the FP phenotype is small, or if the degree of multiple infection of occlusion body-producing cells is not high enough. There are some data available that allow us to obtain rough estimates of W , the replicative advantage. NPV can be maintained in serial passage in insect larvae by injecting haemolymph from infected into uninfected individuals. By avoiding the occlusion body stage, this procedure removes the selective disadvantage of the FP mutant, which should increase in frequency at a rate determined by its replication advantage. Potter *et al.* (1978) found that a TnNPV FP mutant increased in frequency in *Trichoplusia ni* from 24% to 95% in four passages, while Fraser & Hink (1982) found that an FP mutant of GmNPV in *Galleria melonella* increased from 0.7% to 25.9% after two passages. Applying equation (3) this gives values of W of 2.79 and 7.04 respectively. If the first value is typical of the replication advantage of FP mutants then the conditions for polymorphism are quite stringent (see figure 3), while the second value makes polymorphisms much more likely. Clearly more data are needed here.

One of the chief concerns about the release of engineered baculovirus insecticides is the possibility that recombination might occur between the released virus and a wild baculovirus present in the insect population, so that the engineered gene could be transferred to the wild population. The likelihood of such an event taking place is not known and is very difficult to evaluate. However, we note that the conditions required for co-occlusion of different viral genotypes, in particular that occlusion body-producing cells must be infected by more than one nucleocapsid, must also be satisfied for viral recombination to take place. Modifications of the models developed here can be constructed to study the dynamics of recombination in natural populations, although more data is required than is currently available to make this worthwhile. It is clear, though, that key parameters in such a model will be those found here to have a major effect on the dynamics of occlusion-negative genotypes and FP mutants. We suggest that laboratory and field cage experiments studying the dynamics of occlusion-negative genotypes and FP mutants in the context of the models developed here may be used to obtain estimates of these crucial parameters, and so help us understand the risks of releasing genetically-manipulated baculoviruses into the environment.

Other studies of viral evolutionary dynamics have found results that are related to ours. Defective interfering particles are mutant viruses that can only replicate in the presence of wild-type forms, whose

efficiency they reduce. Models of their dynamics have demonstrated the importance of the numbers of viruses infecting a cell (multiplicity of infection) in determining persistence and equilibrium frequencies (Bangham & Kirkwood 1990; Szathmary 1992, 1993; Berry & Nowak 1994; Kirkwood & Bangham 1994). Defective interfering particles may be important in baculovirus biology: it has been suggested that mutants related to FP may have interfering properties leading to persistent, vertically transmitted infections (Burand *et al.* 1986; Fuxa 1993; Goulson & Hauxwell 1995). The multiplicity of infection also is important in determining the relative advantage of unitary and subdivided genomes (Nee 1987). Beyond virology, the models analysed have parallels in those used to study virulence and conflict in parasitology (see, for example, Frank (1994); Nowak & May (1994)) where the probability of superinfection has a major influence on predicted phenotype. These models frequently assume a trade-off between individual fitness and the effects of the parasite on group fitness through damage to the host. In baculoviruses, there may be a trade-off between speed of replication and either the harm done to the host or damage to the cell or occlusion body (the latter just affecting viruses present in the same cell). Analysis of this trade-off may help explain aspects of virus phenotype such as the time taken to kill the host or cell.

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