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Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: a test of the mass action assumption with an insect pathogen

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SUMMARY

Central to theoretical studies of host-pathogen population dynamics is a term describing transmission of the pathogen. This usually assumes that transmission is proportional to the density of infectious hosts or particles and of susceptible individuals. We tested this assumption with the bacterial pathogen *Bacillus thuringiensis* infecting larvae of *Plodia interpunctella*, the Indian meal moth. Transmission was found to increase in a more than linear way with host density in fourth and fifth instar *P. interpunctella*, and to decrease with the density of infectious cadavers in the case of fifth instar larvae. Food availability was shown to play an important part in this process. Therefore, on a number of counts, the usual assumption was found not to apply in our experimental system.

1. INTRODUCTION

That infectious diseases can have profound effects on the population dynamics of their hosts has been demonstrated in a series of theoretical studies beginning with the work of Anderson & May (1979, 1981) and including many more recent studies (Hochberg 1989; Bowers & Begon 1990; Onstad & Carruthers 1990; Hochberg & Waage 1991; Begon *et al.* 1992; Bowers *et al.* 1993; Dwyer 1994; Briggs & Godfray 1995). This work has occurred against a background of very few empirical studies, and many of the basic assumptions upon which these models rest remain unproven. It is clearly vital to our understanding of the importance of pathogens in ecological populations and communities that these assumptions be tested experimentally, so that the theory can be assessed and, if necessary, refined. Insect host-pathogen systems are particularly suitable for experimental manipulation, and we present the results of a test of one of the more important assumptions in most host-pathogen models using one such system.

This assumption is that the rate of transmission of a pathogen is proportional to the density of susceptible hosts and to the density of the infectious units of the pathogen, which is found in most models of host-pathogen population dynamics as a term describing how individuals from the susceptible population pass into the infected population, usually expressed in the form of:

$$\text{number of new infections} = \beta XY,$$

where β, the transmission coefficient, is a constant representing the probability of a new infection arising per contact between a susceptible host and an infectious unit, X is the density of susceptible hosts and Y the density of infectious units, whether these be infected individuals, free living infectious stages or infectious cadavers.

This relation, which is often called the mass action term, has come under some scrutiny: a few theoretical studies have investigated the consequences of relaxing this assumption (Anderson 1979*a*, *b*; Liu *et al.* 1986; Hochberg 1991*a*), another has used a more complex transmission term in an attempt to make a model more realistic (Briggs & Godfray 1995), but empirical tests of the mass action term have been rare. Dwyer (1991) attempted to test the assumption explicitly in a field experiment with the Nuclear Polyhedrosis Virus (NPV) of *Orgyia pseudotsugata*, the Douglas-fir tussock moth, but was unable to draw any clear conclusions regarding the effects of density because of the overriding influence of larval age. Hochberg (1991*b*) found that risk of infection in broods of *Pieris brassicae* infected with a granulosis virus (GV) was related to host age and the number of initial infecteds, but not to the density of susceptible hosts. Dwyer & Elkinton (1993) suggested that a reason for some predictions of the dynamics of NPV epizootics in Gypsy moth, *Lymantria dispar*, being inaccurate was higher NPV transmission at low larval densities, and D'Amico *et al.* (1995) demonstrated that the transmission coefficient of *L. dispar* NPV did indeed decline as the densities of both healthy larvae and pathogen increased. Ebert (1995) found that transmission of a microsporidian, *Pleistophora intestinalis* in *Daphnia magna* did not decrease as rapidly as expected as the volume of experimental containers was increased, which was attributed to swarming behaviour on the part of the host increasing the effective density of hosts and pathogen.

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We describe a test of the mass action term performed by measuring the transmission coefficient of the bacterial pathogen *Bacillus thuringiensis* infecting larvae of the Indian meal moth, *Plodia interpunctella* at varying densities of both susceptible hosts and infectious cadavers. If transmission is accurately described by the mass action term, then estimates of the transmission coefficient should remain constant at all densities of susceptible hosts and of infectious units, which in this case are cadavers of larvae killed by the pathogen. If estimates of the transmission coefficient alter with changes in these densities, this demonstrates that the rate of transmission is changing, and therefore that the simple mass action term is not sufficient to model transmission accurately in these systems.

Of all the parameters used in models of host-pathogen systems, the transmission coefficient is often said to be the most difficult to measure (Anderson & May 1981). Despite this, some attempts to obtain estimates for this key parameter have been made. Techniques that have been used to obtain estimates from field data on vertebrate diseases have been summarized by Hone *et al.* (1992). The detailed records of disease prevalence necessary for this approach are rarely available for diseases of insects (but, see Hochberg & Waage 1991) and direct experimental measurement has been the most widely used technique to obtain estimates in these cases (Dwyer 1991; Dwyer & Elkington 1993; Goulson *et al.* 1995; Thomas *et al.* 1995).

We use a modification of the method first proposed by Dwyer (1991). Transmission rates within a single cycle of infection are measured, giving rise to a reduced, within-generational model of the form

$$\frac{dX}{dt} = -\beta' XY,$$

where Y is the number of infectious units, X is the number of susceptible hosts and β' is the transmission coefficient. β' is used here instead of the more usual β (in the case of close contact transmission) or ν (in the case of free living infective stages) because transmission of the pathogen discussed here appears to be largely by means of susceptible larvae feeding on the cadavers of infected larvae, and neither of the two usual symbols is therefore appropriate.

This equation can then be integrated within limits to obtain:

$$\beta' = \ln\left(\frac{X_0}{X_t}\right)\Big/ Yt,$$

and the transmission coefficient can then be estimated from the initial number of susceptible individuals (X_0) and the number of uninfected individuals remaining at the end of the experiment (X_t).

There are three conditions set by the assumptions behind this model. First, there should be no secondary transmission. In other words all new infections should arise from the initial primary infecteds introduced into an experiment. This condition was met by running the experiments for less time than a newly infected larva takes to become infectious. Second, the amount of infectious material present should be known and not change during the course of the experiment. Third, the mortality rate caused by factors other than the pathogen should be negligible. Both of these latter conditions are tested in this study.

2. THE EXPERIMENTAL SYSTEM

P. interpunctella, a pyralid moth, is a widespread cosmopolitan pest of stored foodstuffs, including dried fruit and grain (Cox & Bell 1991). Cultures were maintained at 28 °C with a 16 h:8 h light-dark cycle, as were all experiments. Under these conditions one generation takes roughly 28 days, and there are five larval instars (Sait *et al.* 1994).

B. thuringiensis (Berliner) is a Gram positive, spore forming bacterium. During sporulation a proteinaceous toxin crystal is formed within the bacterial cell wall. When this is eaten by an insect the toxin is digested in the alkaline conditions of the midgut, and if the dose is sufficient and the insect is of a susceptible species it is killed, often by a combination of the action of the toxin and septicaemia arising from the germinating spores. *B. thuringiensis* is an extremely diverse organism, and there are many subspecies which are specific to particular groups of insects and other invertebrates in terms of their pathogenic effects (Krieg 1987). For these experiments, *B. thuringiensis* var. Kurstaki serotype HD1 was used, obtained from the commercial preparation Dipel. If sufficient spores of this subspecies are ingested, a larva of *P. interpunctella* can be killed in 14 h (R. Knell, unpublished data). Epizootics of *B. thuringiensis* have been reported as occurring in populations of *P. interpunctella* in stored grain facilities (Dulmage & Aizawa 1982), and previous work has suggested, as mentioned above, that transmission is largely by cannibalism of infected cadavers, as the larvae do not appear to lyse after death (Burges & Hurst 1977), as is thought to be the case with many other insect pathogens.

3. EXPERIMENTAL METHODS

Experimental larvae were produced by adding approximately 200 eggs from an outbreeding stock to 200 g of medium consisting of 10:1:1 wheat bran: brewers' yeast: glycerol. Early fourth and fifth instar *P. interpunctella* larvae were obtained from these cultures, and placed in 50 ml screw-top glass jars with either 50 mg or 100 mg of food. The amount of food was deliberately limited to allow for ease of counting and manipulation of larvae. The uninfected larvae were then kept for 36 h, to eliminate any effects from handling. Any larvae that died during this period were replaced from equivalent jars randomly included in the experiment for this purpose.

Infected cadavers were obtained by droplet dosing (Hughes & Wood 1981) fourth or fifth instar larvae with a 40 mg. ml^{-1} solution of Dipel 24 h before the start of the experiment, a concentration sufficient to cause greater than 95% mortality. Four experiments were done.

1. To determine whether the amount of infectious material changed over the course of the experiments,

Table 1. *Mortality caused by* B. thuringiensis *in each experimental treatment*

experiment	treatment	mortality	mean proportional deviation	standard deviation
1 (effect of time)	time (hours)			
	4		0.065	0.058
	8		0.135	0.168
	12		0.125	0.089
	16		0.245	0.109
2 (effect of host density)	instar	density of hosts		
	4	5	0.060	0.135
		10	0.090	0.129
		15	0.200	0.153
		20	0.265	0.167
	5	5	0.140	0.190
		10	0.470	0.211
		15	0.608	0.165
		20	0.625	0.140
3 (effect of density of infected cadavers	instar	density of infected cadavers		
	4	2	0.110	0.110
		4	0.120	0.092
		6	0.140	0.097
		8	0.180	0.169
		10	0.210	0.137
	5	2	0.370	0.189
		4	0.367	0.265
		6	0.500	0.183
		8	0.540	0.150
		10	0.510	0.203
4 (effect of host density and food availability)	host density	quantity of food available (mg)		
	10	50	0.140	0.070
	10	100	0.050	0.053
	20	50	0.325	0.134
	20	100	0.165	0.133

20 4th instar larvae were placed in jars with 50 mg of food. After 36 h, two infectious 4th instar cadavers were added for four, eight, 12 or 16 hours, after which time all the susceptible hosts were removed, placed individually in cells of 5×5 divided Petri dishes and excess food added.

2. To investigate the effects of the density of susceptible hosts on transmission, five, ten, 15 or 20 randomly selected healthy larvae were placed into each jar. After 36 h, two infectious cadavers were added for 12 h, after which time all susceptible hosts were removed individually into excess food as in experiment 1. This experiment was done with both 4th and 5th instar larvae, the 4th instar larvae being provided with 50 mg of food, the 5th instars with 100 mg.

3. To investigate the effects of the density of infected cadavers on transmission, ten healthy 4th or 5th instar larvae were used in each jar and after 36 h two, four, six, eight or ten infected cadavers added for 12 h, after which time susceptible hosts were removed into excess food as in the first experiment. As in the previous experiment, 4th instars had 50 mg of food and 5th instars had 100 mg.

4. To investigate the importance of food availability, and any interaction between this and the density of susceptible hosts in determining the transmission coefficient, ten or 20 susceptible 4th instar larvae were placed in jars, half of each density treatment being given 50 mg and half 100 mg of food. After 36 h, two infected cadavers were added for 12 h, and susceptible hosts then removed into excess food as above.

Larvae were monitored 48 h after the end of each experiment for signs of infection. Those that had acquired a lethal dose of *B. thuringiensis* were easily distinguished by their black colour. Any mortality not caused by the disease was also recorded. Each experiment was replicated ten times.

4. RESULTS

Non-disease mortality was 1.6% in experiment 1, 2.3% in experiment 2, 1.8% in experiment 3 and 2.0% in experiment 4. Removal of all replicates for which there was 10% or more non-disease mortality had no effect on the results of any of the analyses, so the assumption that this is negligible appears reasonable.

Table 1 gives the mean proportional mortalities arising from the pathogen in each experimental treatment. These were used to calculate values for β' as described in §3, and each experiment will now be considered individually. The results from experiment 1 are shown in figure 1. If the amount of infectious material was reduced during the course of the

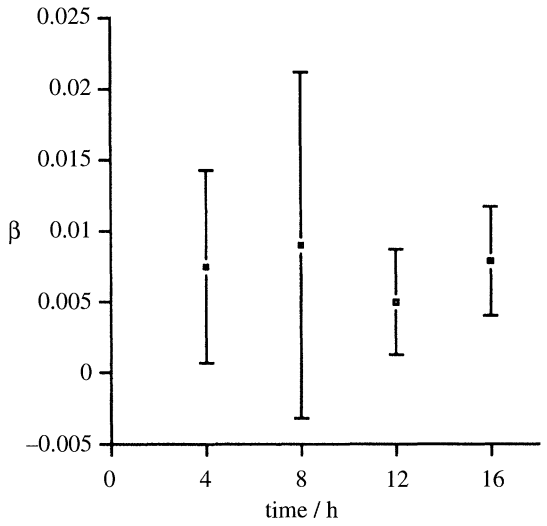

Figure 1. Results from experiment 1. Relation between the transmission coefficient and time. Error bars = 95% confidence limits.

experiment, it would be expected that the estimates of the transmission coefficient would fall as the experiment continued. There is no significant change in the estimate of the transmission coefficient with increasing time (ANOVA F-test, $p = 0.680$), indicating that the condition that the amount of infectious material should remain constant throughout the experiment is met. This finding is consistent with that expected from visual observation of the infectious cadavers, which could be seen to be largely intact even towards the end of the experiments. Figure 2 shows the estimates of the transmission coefficient obtained with the differing densities of susceptible hosts and cadavers.

In the case of fifth instar *P. interpunctella* larvae, the transmission coefficient increased with susceptible host density (ANOVA F-test, $p < 0.001$) and decreased with density of infectious individuals (ANOVA F-test, $p = 0.003$). Transmission increased with the density of susceptible fourth instar larvae (ANOVA F-test, $p = 0.024$), and although there was a suggestion of a decrease with cadaver density in fourth instar larvae this was not significant (ANOVA F-test, $p = 0.128$). In those cases where the factor had a significant effect, an orthogonal polynomial trends analysis was used to determine the shape of the relationship. This is an analysis which determines the simplest polynomial which fits the data (for details, see Winer *et al.*, (1991)). The results of these analyses are summarized in table 1. Although there was a strong suggestion of non-linearity in some of the data, particularly those for changes in

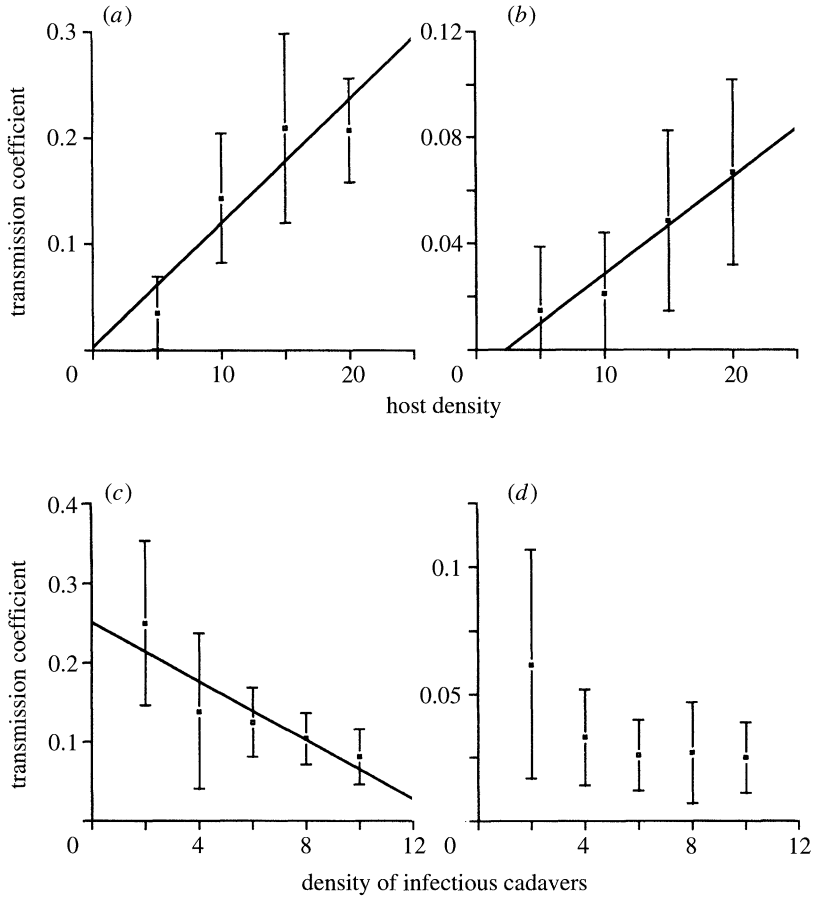

Figure 2. Results from experiments 2 and 3. (*a*) Relation between density of susceptible hosts and transmission coefficient in 5th instar larvae (regression: $y = 0.012x + 0.003$). (*b*) Relation between density of susceptible hosts and transmission coefficient in 4th instar larvae (regression: $y = 0.004x - 0.008$). (*c*) Relation between density of infectious cadavers and transmission coefficient in 5th instar larvae (regression: $y = -0.019x + 0.252$). (*d*) Relation between density of infectious cadavers and transmission coefficient in 4th instar larvae. All error bars = 95% confidence limits.

Table 2. *Results of orthogonal polynominal trends analysis*

(*a*) 5th instar larvae, effect of susceptible host density

order of polynomial	MS error	SS trend	*F*	*p*
1st	0.0063	0.169	26.99	< 0.01**
2nd	0.0063	0.030	4.792	> 0.05
3rd	0.0063	0.000	0.043	> 0.05

(*b*) 5th instar larvae, effect of density of infectious cadavers

order of polynomial	MS error	SS trend	*F*	*p*
1st	0.0090	0.139	15.51	< 0.01**
2nd	0.0090	0.015	1.674	> 0.05
3rd	0.0090	0.010	1.083	> 0.05

(*c*) 4th instar larvae, effect of susceptible host density

order of polynomial	MS error	SS trend	*F*	*p*
1st	0.0017	0.017	10.24	< 0.01**
2nd	0.0017	0.001	0.459	> 0.05
3rd	0.0017	0.000	0.280	> 0.05

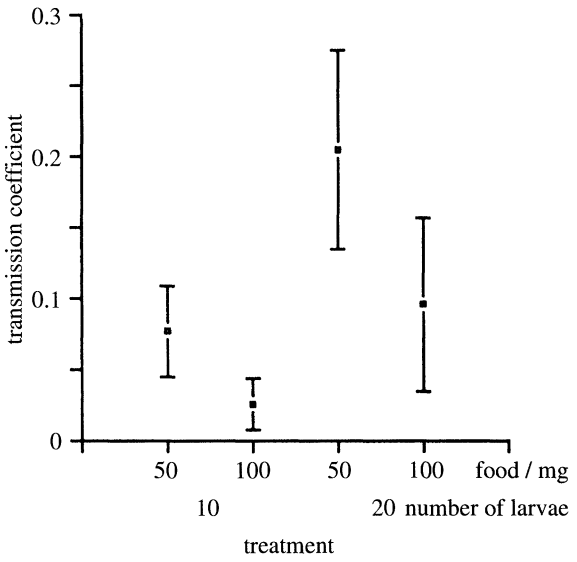

Figure 3. Results from experiment 4. Transmission coefficient measured at different host densities and food availabilities. Error bars = 95% confidence limits.

transmission coefficient with density of fifth instar hosts, none of the relations differed significantly from a straight line. The age of the larvae also had a strong effect. There was substantially more transmission in fifth instar larvae than fourth instars, leading to a higher estimate for the transmission coefficient in the older larvae.

The results from the food availability experiment are presented in figure 3. Both host density and food availability had highly significant effects on the transmission coefficient (two-way ANOVA, host density: $p < 0.001$, food availability: $p = 0.001$, interaction: $p = 0.193$). Increasing food led to lower estimates and increasing host density led to higher estimates. The interaction term was non-significant.

5. DISCUSSION

This is the one of the first studies to test the mass action assumption in an insect pathogen, and we have demonstrated that it is inadequate to describe transmission dynamics in our experimental system. There are changes in the transmission rate with variation in the density of susceptible hosts, and also in the response to the density of fifth instar cadavers. Age of host larvae is also important, both in determining the magnitude of the transmission coefficient and also in determining the slope of the changes with host and cadaver densities.

Changes in the magnitude of the transmission coefficient with host age have been discussed by Dwyer, (1991) and Goulson, *et al.* (1995), but differences in the response of the transmission coefficient to host or infective density resulting from the age of the hosts in question has not been demonstrated before. The possible influence of such changes has not been addressed in any theoretical studies of which the authors are aware, but the implications for studies of age structure and disease dynamics may be profound.

The transmission coefficient is analogous to the searching efficiency of a parasitoid (Hassell & Anderson 1989), and the mass action term is the equivalent of a type 1 functional response (see Antonovics *et al.* 1995 for further discussion of this). Whereas in the case of a parasitoid or predator any deviation from a linear response to host density is normally seen as determined by behavioural changes on the part of the searching parasitoid, in the case of pathogens any such deviations are expected to result from changes in either behaviour or physiological status on the part of the host. That such an unequivocal response to host density should be found with *B. thuringiensis* is perhaps not surprising, given that transmission of the pathogen is dependent on an aspect of host behaviour which might be expected to change considerably with the density of susceptible hosts, namely the cannibalism of infectious cadavers. The critical importance of food availability in determining transmission rates has also been demonstrated. This therefore appears to be the driving force behind the behavioural changes with density that lead to a change in transmission, rather than, for instance, increased aggression resulting from increased contacts with conspecifics.

There are many other changes that occur with host density that could contribute to a change in transmission rate in other systems. Changes in dispersal or movement rate could lead to changes in the contact rate between healthy and infected hosts, in the case of close-contact transmission, or to changes in the contact rate with free-living infective stages. Density-dependent changes in behaviour governing interactions with conspecifics could lead to longer, shorter or physically closer encounters between infected and susceptible hosts, and therefore a change in the probability of transmission of a close contact disease.

Physiological changes that affect the likelihood of transmission are mostly related to food quality and availability. Starvation has important effects on the susceptibility of insects to disease (Steinhaus 1958).

Changes in food quality could also be important: feeding tends to lead to host plant responses including increased levels of resins, fibre and toxins (Crawley 1983), which could all have effects on the susceptibility of a herbivore to disease. There are also cases where feeding can lead to an increase in food quality, as in the case of *Eucalyptus blakelyi* defoliated by the sawfly *Perga affinis affinis* (Carne 1965, cited in Crawley 1983), which could have the opposite effect on host susceptibility to disease. Changes in host plant, the age of leaves eaten, and the amount of various vitamins, protein, nitrogen or carbohydrate have all been shown to have effects on the susceptibility of insects to pathogens (Watanabe 1987), and D'Amico *et al.* (1995) suggest that one reason for the decline in transmission coefficent with host density observed with *L. dispar* NPV is changes in larval susceptibility to the virus arising from defoliation.

Theoretical studies (Hochberg 1991*a*) have suggested that increases in the transmission rate with susceptible host density would tend to stabilize a host-pathogen system. However, *B. thuringiensis* is not known to exist in long-term stable relations with its hosts, and accounts of its appearance in laboratory insect cultures and in infested stored grain facilities suggest that it often appears in sudden, devastating epizootics (Burges & Hurst 1977; Dulmage & Aizawa 1982). This suggests that unstable dynamics in this case arise from events which are occurring on a different timescale to that of the snapshot of the host pathogen dynamics which we are considering here. A scenario can be envisaged, for example, in which the host populations in question are normally maintained below the threshold density for pathogen transmission, and occasionally rise to levels considerably higher. A widespread epizootic could result which would then die out as the host population was reduced below the threshold density. Increases in transmission rate with host density would tend to exaggerate the transition between absence of disease and epizootic.

A decline in the estimated transmission coefficient with increasing density of infectious cadavers is found in the system, albeit only to a statistically significant degree in fifth instar larvae. Hochberg (1991*a*) points out that it is reasonable to expect this pattern because the risk of a healthy host contracting a disease cannot increase indefinitely with the density of infectious hosts. There must be a point where all of the susceptible hosts become infected, so that increases in the density of infectious units do not lead to any increase in transmission. This type of effect is probably most important in small populations.

Even when the numbers becoming infected are small compared with the population of susceptible hosts, any variability in the probability of transmission between healthy and diseased hosts can lead to this type of response. For example, consider a situation whereby some individuals in the population of susceptible hosts are more likely to contract the disease in question than others. A single infectious unit would then infect a greater proportion of the 'more at risk' class than of the 'less at risk' class (assuming one infectious unit is able to infect more than one host), thus reducing the fraction of the susceptible population in the 'more at risk' class available for infection by a second infectious unit. A decline in transmission coefficient would then be seen with increasing densities of infectious units. Variability in the probability of transmission can arise from variabilities in the rate of contact with infectious particles, which can be generated by spatial heterogeneity (Briggs & Godfray 1995), or from variability in the chance of transmission per contact. Again, a decline in transmission rate with density of infectious hosts should stabilize a host-pathogen interaction.

The question remains as to what a suitable transmission term would be in modelling these systems. Whereas we have demonstrated that in the mass action assumption is inadequate to describe transmission in this particular system, it is difficult to generalize because of a lack of similar studies of other host-pathogen systems, and more work is clearly necessary.

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