

Host behaviour and exposure risk in an insect–pathogen interaction

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Summary

1. Studies of variability in host resistance to disease generally emphasize variability in susceptibility given exposure, neglecting the possibility that hosts may vary in behaviours that affect the risk of exposure.
2. In many insects, horizontal transmission of baculoviruses occurs when larvae consume foliage contaminated by the cadavers of virus-infected conspecific larvae; so, host behaviour may have a strong effect on the risk of infection.
3. We studied variability in the behaviour of gypsy moth (*Lymantria dispar*) larvae, which are able to detect and avoid virus-contaminated foliage.
4. Our results show that detection ability can be affected by the family line that larvae originate from, even at some distance from a virus-infected cadaver, and suggest that cadaver-detection ability may be heritable.
5. There is thus the potential for natural selection to act on cadaver-detection ability, and thereby to affect the dynamics of pathogen-driven cycles in gypsy moth populations.
6. We argue that host behaviour is a neglected component in studies of variability in disease resistance.

Key-words: evolution of resistance, gypsy moth *Lymantria dispar*, host pathogen interactions, nucleopolyhedrovirus, probability of consumption

Introduction

For natural selection to act on disease resistance, hosts must vary in their susceptibility to a disease (Gillespie 1975), and so variability in disease resistance is a widely studied phenomenon (Kraaijeveld, Van Alphen & Godfray 1998). Most studies, however, focus on variability in internal susceptibility, considering only factors that affect the risk of infection given exposure, rather than factors that affect the risk of exposure (Woolhouse *et al.* 2002). For animal diseases, this approach neglects the possibility that hosts may vary in behaviours that affect their exposure risk. Although behaviour clearly affects infection risk in some species (Anderson & May 1992; Kiesecker & Skelly 2000; Tarpay 2003; Evans *et al.* 2006), for many others it is difficult to even identify behaviours that affect exposure risk. Tests of the heritability of host behaviours that affect infection risk are therefore, to our knowledge, non-existent.

Baculoviruses of insects are an obvious case in which host behaviours are likely to affect exposure risk. Baculoviruses of

many insects are transmitted when host larvae consume foliage contaminated with virus-infected cadavers (Cory & Myers 2003). Variability in insect feeding behaviour can therefore have a strong effect on infection risk, in some cases equalling the effects of variability in innate susceptibility (Dwyer, Firestone & Stevens 2005). In the case of the gypsy moth (*Lymantria dispar* L.) that we study here, previous work has shown that larvae can detect and avoid virus-infected cadavers (Capinera, Kirouac & Barbosa 1976). We therefore use the protocol of Capinera *et al.* (1976) to show that gypsy moth larvae vary in their cadaver-avoidance behaviour, that this variability appears to be heritable and that small-scale spatial structure can affect the behaviour. Our work provides a clear example of heritable variation in a behaviour that affects risk of disease infection.

Insect baculoviruses are also of economic importance. Outbreaks of forest defoliators can lead to growth reductions and tree death in commercially valuable timber, but in many species the resulting economic losses would be even more severe if outbreaks were not terminated by baculovirus epidemics (Liebhold & Kamata 2000; Moreau & 2007). More generally, baculoviruses constitute the primary pathogens of

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many forest lepidoptera (Myers 1993), and identifying the mechanisms driving baculovirus spread is important for understanding the population dynamics of these insects (Anderson & May 1980; Bowers, Begon & Hodgkinson 1993; Dwyer, Dushoff & Yee 2004).

Materials and methods

As in many insects, gypsy moth larvae that are infected with their baculovirus release infectious particles known as 'occlusion bodies' shortly after death, and the occlusion bodies are then available to infect additional larvae (Cory & Myers 2003). A standard method of studying baculovirus transmission is therefore to feed a solution of occlusion bodies to host larvae in the laboratory (Cory & Hoover 2006). In this type of experiment, however, larvae that do not consume the entire dose are discarded; so, there is no allowance for the effects of behaviour. An alternative method is therefore to allow uninfected larvae to consume virus-contaminated foliage in the field (D'Amico *et al.* 1998; Hails *et al.* 2002). Field transmission studies, however, have the opposite difficulty of standard methods, in that they do not allow us to disentangle behaviour from susceptibility.

We therefore measured behaviour in the laboratory. We presented healthy fourth-instar gypsy moth larvae with two leaf discs, a virus-free disc and a disc contaminated with a virus-infected cadaver, and we quantified cadaver-avoidance behaviour. Specifically, we tested for effects of family and distance of the leaf disc from the cadaver on the difference in the amount eaten between the two types of disc.

To produce foliage contaminated with virus-infected cadavers we fed a virus solution to hatchling larvae at a dose sufficient to ensure 99% mortality (Dwyer *et al.* 2005). We then placed these infected individuals on the foliage of red oak trees in the field. To keep the larvae from escaping, we enclosed the branches in mesh bags that allow the passage of air, water and much of the natural spectrum of light. Larvae were left on the leaves for 5 days, to ensure that they were dead, and then were brought into the laboratory. We then used corkborers to make leaf discs of approximately 1 cm² in area that contained cadavers, and for controls we made leaf discs from uncontaminated foliage taken from adjacent branches of the same trees.

Pairs of leaf discs with similar vein structures, one contaminated and one clean, were matched and photographed. The trees used in this study were the same as those used in Elderd, Dushoff & Dwyer (2008), which had a level of natural contamination that was effectively zero (less than 0.8% of larvae on control foliage in Elderd *et al.* 2008 became infected, and anecdotal evidence suggests that these few infections were due to handling in the laboratory). In our first year of trials (2006), we left the clean foliage uncovered by mesh bags. In our second year (2007), however, both control and virus-contaminated leaves were placed inside bags to ensure that differences in foliage quality between the two disc types did not alter larval preferences. In both years, experiments were conducted in July and August when foliage quality is relatively constant both chemically and physically (Hunter & Lechowicz 1992; Salminen *et al.* 2004), and herbivory at the field site was nearly zero (G. Dwyer, personal observation). It therefore seems unlikely that the observed preference for uncontaminated foliage in the first year was due to the lack of mesh bags on the control foliage compared with the experimental foliage, especially given that the experimental foliage was bagged for only 5 days. More concretely, as we document in the Results section, levels of cadaver avoidance in the second year of trials were indistinguishable from levels in the first year. We therefore include data from both years, and we attribute differences in consumption between the two leaf disc

types to the presence or absence of virus particles rather than to some other factor.

Note that Capinera *et al.* (1976) painted leaves with a slurry of virus-infected cadavers in water, whereas we allowed cadavers to die on leaves naturally. Cadavers consist of high concentrations of infectious particles in viscous patches (D'Amico *et al.* 2005). Foliage contaminated with infectious cadavers is therefore likely to have a different consistency than the contaminated foliage used by Capinera *et al.* In nature uninfected larvae encounter infectious cadavers, and so by using cadavers that died directly on leaves we approximated natural virus transmission more closely.

To produce uninfected larvae, we hatched larvae from egg masses that had been soaked for 90 min in 10% formalin, which effectively surface sterilizes the eggs (Dwyer & Elkinton 1995). Feral strain insects came from egg masses collected near Gladwin, MI (44° N, 84.5° W). Laboratory strain insects were hatched from a strain that has been maintained by the USDA for many generations, and which are consequently of lower heterogeneity than feral insects (Dwyer, Elkinton & Buonaccorsi 1997). All healthy larvae were reared to the fourth instar, and then were used in experiments. To ensure that uninfected larvae were developmentally synchronized, we used only larvae that had moulted to the fourth instar in the previous 48 h (Grove & Hoover 2007).

We then presented each healthy larva with a matched pair of leaf discs, one virus contaminated and one clean. These larvae were allowed to feed overnight, and were then removed to cups of artificial diet. We photographed the leaf discs before and after feeding, and we quantified the difference between the prefeeding and post-feeding leaf areas using image software (IMAGEJ, <http://rsbweb.nih.gov/ij/>).

We carried out several experiments using this protocol. In 2006, we tested for effects of full-sibling families on cadaver avoidance by hatching each family from a single feral egg mass. We performed two initial trials using the feral strain, one with 10 families of 11 individuals each and the other with 10 families of 25 individuals each. We then performed a third trial using eight families of 25 individuals each, using the laboratory strain.

With full-sibling experiments, differences among families could hypothetically be the result of environmental differences rather than genetic differences. For example, although larvae were reared under identical conditions in the laboratory, it is possible that differences among families were due to the effects of variability in resource quality among female parents in the previous generation. Such variability could affect the susceptibility of offspring, a phenomenon known as a 'maternal effect' (Myers 2000). One way to disentangle maternal effects from genetic effects is to mate males to multiple females, and then to test for effects of sire independently of the effects of dam (Lynch & Walsh 1998). In 2007, we therefore mated individual, feral, adult male gypsy moths to two or three feral dams per male, to produce half-sibling groups. We tested 10 half-sibling groups, each with the same sire and two or three dams, with 28–127 individuals in each group.

In 2006, we also tested for effects of spatial structure on detection ability. Full-sibling feral larvae were given a choice between a clean leaf disc and a disc that was tangent to, but did not include, a cadaver-covered leaf disc (see Fig. 1). This allowed us to determine whether virus particles that leak out of a cadaver can be detected and avoided as much as 1 cm away from the cadaver, allowing us to roughly quantify the spatial scale over which avoidance behaviour occurs. We again kept track of full-sibling families, using six families of 25 individuals each.

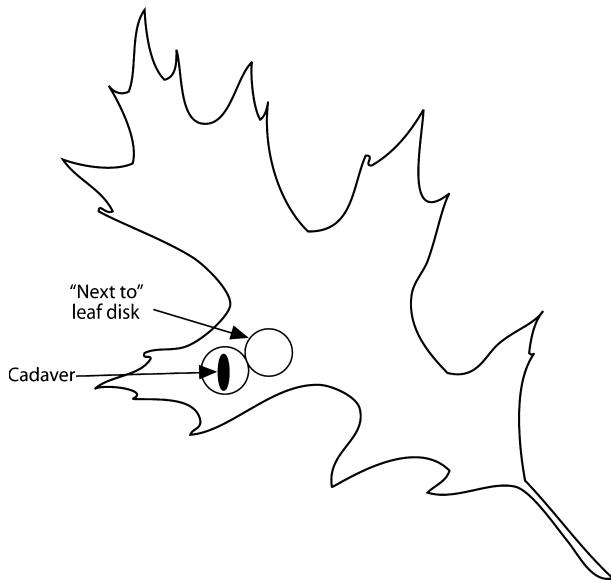


Fig. 1. In the spatial structure experiment, individuals were presented with a choice between a clean leaf disc and a disc that was next to, but did not include, a cadaver-infected leaf disc.

We intentionally designed our experiments to test for variability in behaviours that affect infection risk, rather than to test for effects of variability in behaviour on infection risk itself, for several reasons. First, we were only able to measure total area consumed, whereas risk of infection is also affected by how close a larva gets to a cadaver while it feeds. Second, we could not control for variability in physiological susceptibility independently of behaviours that affect exposure, yet variability in physiological susceptibility in gypsy moth larvae is known to be quite high (Dwyer *et al.* 1997). Larvae that ate similar areas of contaminated foliage may therefore have had very different infection risks. We therefore did not expect that our experiments would provide much evidence for effects of behaviour on infection risk. However, in three trials (the two trials using full-sibling feral insects and the trial using a laboratory strain), we nevertheless reared larvae individually on artificial diet for several weeks after exposure to determine which larvae had become infected. The resulting data did indeed show that the amount of leaf area consumed can affect infection risk, but they also showed no effects on infection risk of interactions between family and area eaten, as we expected. It is thus in turn difficult to demonstrate that heritability in cadaver-detection ability alters infection rates. These data are tangential to the main thrust of our work, and so they are presented as Supporting Information.

STATISTICAL METHODS

As we have described, Capinera *et al.* (1976) provided strong evidence that gypsy moths can detect cadavers. Our main goal was instead to test whether cadaver-detection ability is heritable. Statistically, this meant testing for the effects of either full- or half-sib families on the difference in the amount of foliage eaten between cadaver-contaminated and uncontaminated leaf discs. Some larvae, however, may eat more than other larvae, irrespective of whether a disc is cadaver contaminated or not. The amount of one type of disc that a larvae eats may thus not be independent of the amount that the larva eats of the other type of disc, and it was crucial for our statistical analyses to take this lack of independence into account.

We therefore constructed our statistical models in the following way. In our models, i is the full-sib family, j is the leaf type, 0 for uncontaminated and 1 for cadaver contaminated, and k is the individual larva. For our full-sib experiment, we then write y_{ijk} for the average amount of leaf type j eaten by individual k in family i , which depends on the overall average amount eaten μ and the error term ϵ_{ijk} . In addition, however, we took into account the lack of independence of contaminated and uncontaminated leaf discs that were fed upon by the same larva, which we represent with the term $b_{k(i)}$. Note that the symbol $k(i)$ signifies that individuals are nested within families (Gomez *et al.* 2007). Our simplest statistical model is thus,

$$y_{ijk} = \mu + b_{k(i)} + \epsilon_{ijk}. \quad \text{eqn 1}$$

If this model had fit best, we would have concluded that variability among individuals, including correlations in feeding intensity within individuals, was sufficient to explain our data. The amount eaten, however, may also be affected by the presence of a cadaver, the effect of which we represent with the symbol D_j . We then have that $D_0=0$ for uncontaminated leaves, so that D_1 is the change in the average amount eaten due to the presence of a cadaver. Our next most complicated model is therefore,

$$y_{ijk} = \mu + b_{k(i)} + D_j + \epsilon_{ijk}. \quad \text{eqn 2}$$

If larvae avoided cadavers, as we expected them to, then it should be true that $D_j < 0$. As we will describe, larvae do indeed avoid cadavers, and so we refer to this behaviour as avoidance rather than preference.

We further suspected, however, that there would be an effect of family on feeding behaviour. To equation (2), we therefore added the effects of family F_i on the average amount eaten;

$$y_{ijk} = \mu + b_{k(i)} + D_j + F_i + \epsilon_{ijk}. \quad \text{eqn 3}$$

In the above equation, we have begun by assuming that the effect of family is the same on both types of disc. If in addition we allow for an effect of family on cadaver-detection ability, the effect of family must instead vary between disc types. In our next model, we therefore added the term $F_i D_j$, which represents the interaction between the family effect F_i and the disc-type effect D_j :

$$y_{ijk} = \mu + D_j + F_i + F_i D_j + b_{k(i)} + \epsilon_{ijk}. \quad \text{eqn 4}$$

Note that for uncontaminated discs, $j=0$ and $D_0=0$, reducing the full model to $y_{i0k} = \mu + F_i + b_{k(i)} + \epsilon_{i0k}$. Our statistical approach was thus to test whether a model that included the interaction effect $F_i D_j$, namely equation (4), provided a better fit to the data than did the models that did not include that term, namely equations (1)–(3). We then used the Akaike Information Criterion (AIC) to choose the best model, as we describe in more detail below. We reiterate, however, that we were careful to allow for the lack of independence of discs within a larva, and also that we allowed for direct effects of family and cadaver presence, to test whether direct effects provided a better explanation than the interaction effect which is our main interest. In addition, note that disc is taken to be a fixed effect, while the other variables are taken to be random effects.

In the half-sibling experiment, each individual could also be grouped by sire, and so we added the effects of sire to our models. The response variable is then the amount of foliage of the j th leaf type consumed by the k th individual from the i th family and the l th sire. The effect of sire l on the amount eaten is then S_l , while the interaction effect is $S_l D_j$. The term $S_l D_j$ then represents a difference in cadaver-detection ability between the offspring of different sires, and thus allows cadaver-detection ability to be affected by sire. The full model is then,

$$y_{ijkl} = \mu + D_j + F_{l(i)}D_j + S_lD_j + b_{k(ij)} + \epsilon_{ijkl}. \quad \text{eqn 5}$$

Note that here family i is nested within sire l because families arise from multiple dams that are mated to the same sire. As in the full-sib experiments, we compared this model with simpler models in which we deleted all but the average consumption rate μ and the individual effect term $b_{k(ij)}$. In the case of the half-sibling experiments, our goal was thus to determine whether there was an effect of sire on cadaver-detection ability, and thus whether cadaver-detection ability is heritable, and again we took into account the lack of independence of leaf discs within a larva. Disc is again considered a fixed effect and all other variables are random effects.

The statistical models that we have described are linear mixed effects models, which we implemented using the package 'lme4' in the R programming language (Bates 2007). To choose among the models, we used the AIC. In contrast to tests of statistical significance, the AIC has the advantage that it is based on the assumption that 'all models are wrong, but some models are useful' (Box 1979), and it allows us to choose among multiple models at the same time (Burnham & Anderson 2002). AIC is a useful statistical tool in our case because we are not sure which of our many models will best fit our data. The statistical foundations of AIC analyses, however, are quite different from those of significance tests, and so recommended practice is to only include one type of analysis, not both (Burnham & Anderson 2002). As for our purposes AIC is the best choice, we do not present the results of significance tests.

Akaike Information Criterion scores are calculated according to,

$$\text{AIC} = -2 \log(\mathcal{L}(\hat{\theta}|y)) + 2K \quad \text{eqn 6}$$

where $-2 \log(\mathcal{L}(\hat{\theta}|y))$ is twice the negative log-likelihood of the parameters $\hat{\theta}$ given the data y and K is the number of parameters in the model. The best model is the model with the lowest AIC score. Models with more parameters are likely to provide a better fit, and thus a smaller value of the negative log-likelihood, but they will be penalized by the $2K$ term. The AIC thus operates on the principle of parsimony to find the model that best trades off better fit with less complexity (more precisely, the model with the lowest AIC is the

model that minimizes the distance between that model and the true model, Burnham & Anderson 2002). To compare AIC scores between models, we use the ΔAIC for each model (Burnham & Anderson 2002), which is the AIC score of that model minus the AIC of the best model. The model with the best fit thus has a ΔAIC score of zero. To evaluate the relative strength of evidence for different models, we used AIC weights (Burnham & Anderson 2002), such that, of Z total models, model r has weight:

$$w_r = \frac{\exp(-\frac{1}{2}\Delta\text{AIC}_r)}{\sum_{z=1}^Z \exp(-\frac{1}{2}\Delta\text{AIC}_z)}. \quad \text{eqn 7}$$

The AIC weight for a particular model is thus a measure of the probability that that model is the best model, and so the relative support for different models can be assessed from the weights.

Results

In all of our experiments, models that included the effects of cadavers explained the data much better than models that did not, and in every case, the average effect of a cadaver was to reduce feeding (Table 1). Our experiments thus confirm the work of Capinera *et al.* (1976) that gypsy moth larvae can avoid and detect cadavers. Models that allowed for differences in consumption among families also fit the data much better than models with no family effects, indicating that different families consumed different total amounts of foliage (Table 1). The most interesting feature of our results, however, is that there were interaction effects between family and the presence of a cadaver. That is, families differed in their ability to detect cadavers.

As Fig. 2 shows, average consumption in some full-sibling families was quite low on the cadaver-contaminated discs compared with the uncontaminated discs, whereas in other families average consumption was roughly the same on both types of disc. Note that in AIC analyses, if the second best

Table 1. Akaike Information Criterion analysis of full-sibling experiments

Model	AIC	ΔAIC	AIC weights
Full-sib feral strain trial 1 – (10 families of 11 individuals)			
Individual variation	0.72	132.6	0
+ Presence of a virus disc	-122.4	9.44	0.007
+ Differences among families	-128.5	3.35	0.157
+ Differences in detection by families	-131.9	0	0.836
Full-sib feral strain trial 2 – (10 families of 25 individuals)			
Individual variation	-897.7	155.4	0
+ Presence of a virus disc	-994.5	58.56	0
+ Differences among families	-1050	2.73	0.203
+ Differences in detection by families	-1053	0	0.797
Full-sib laboratory strain – (8 families of 25 individuals)			
Individual variation	-259.8	60.51	0
+ Presence of a virus disc	-319.4	0.83	0.326
+ Differences among families	-320.3	0	0.493
+ Differences in detection by families	-318.3	2	0.181
Spatial Structure – (6 families of 25 individuals)			
Individual variation	-523.9	56.28	0
+ Presence of a virus disc	-568.5	11.7	0
+ Differences among families	-580.2	0	0.669
+ Differences in detection by families	-578.8	1.42	0.329

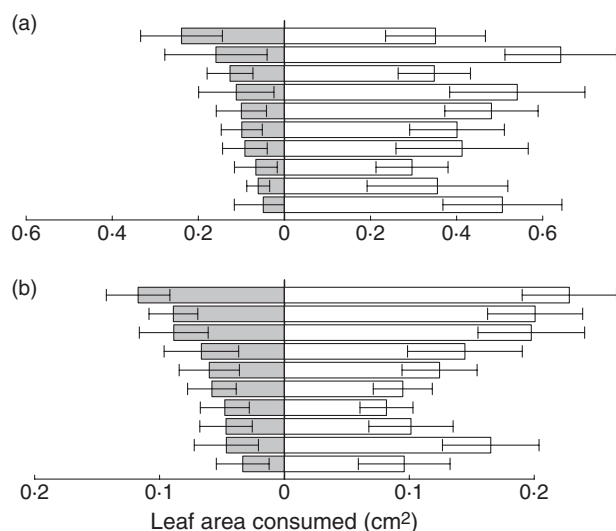


Fig. 2. Each bar represents one family (individuals from the same egg mass). The dark portion of the bar represents the amount of contaminated disc consumed, and the light portion of the bar represents the amount of control disc consumed. (a) and (b) are two different trials, both using full-sibling feral insects.

model has a ΔAIC value less than two, then the data cannot distinguish between that model and the best model. If one or more ΔAIC values are between two and three, support for the best model is only moderately strong; whereas if all values are greater than three, then support for the best model is very strong (Burnham & Anderson 2002). From this perspective, the strength of support for the model with a family effect on detection was only moderately strong in the second experiment, but in both experiments the best model included family effects on cadaver avoidance, strongly suggesting that the effect is real (Table 1). By contrast, the best model for laboratory-reared insects included no variability in cadaver avoidance between families, but that model could not be distinguished from a model that did include variability in avoidance between families. It therefore appears that variability in the ability to detect cadavers is reduced among larvae from the laboratory colony (Fig. 3). Previous studies have similarly found reduced variability among laboratory larvae (Dwyer *et al.* 1997).

Spatial structure is also an important factor influencing virus detection (Table 1). Larvae were able to detect and avoid contaminated leaf discs at distances of approximately 0.5 cm away from the cadavers, as evidenced by decreased consumption of contaminated discs. Cadavers can therefore influence consumption even if there is no visual evidence of a cadaver on a disc (Fig. 4). Moreover, the best model again included an effect of family on overall consumption, and there was some support for a second model that allowed for differences in avoidance by families. This experiment thus provides additional evidence for family effects, although the evidence is not as strong. Note that the data in Fig. 4 were collected on the same day using insects hatched from the same egg masses as the insects that produced the data in Fig. 2b.

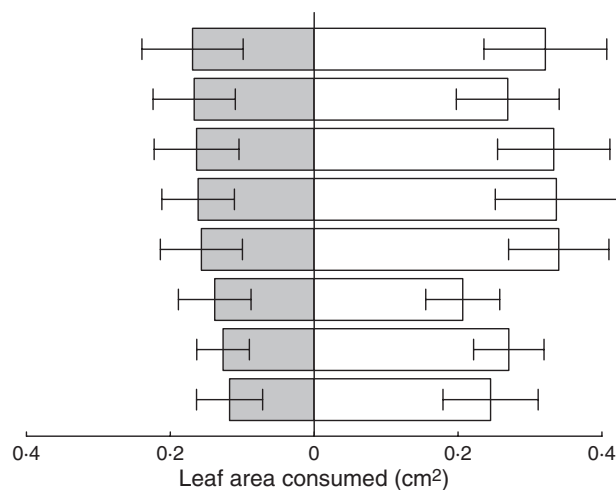


Fig. 3. Full-sibling trial using laboratory-reared insects. These larvae were hatched from a strain that has been maintained by the USDA for many generations, and are thus of lower heterogeneity than feral larvae.

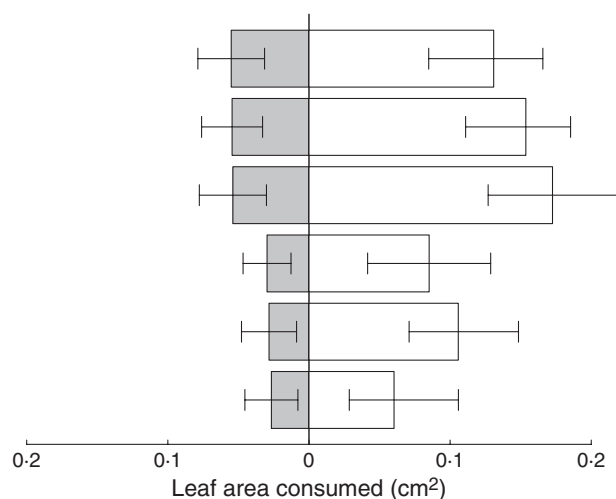


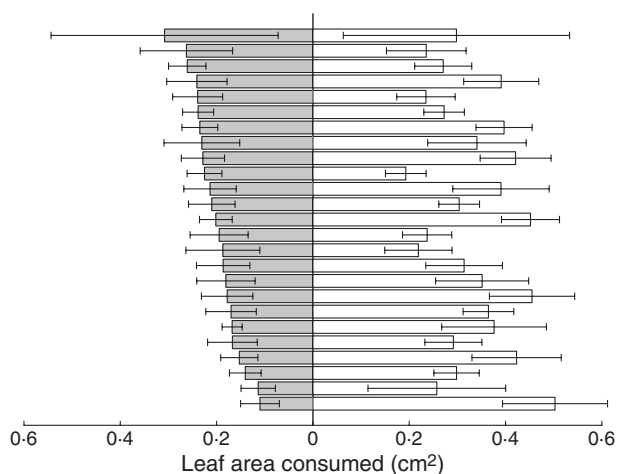
Fig. 4. Full-sibling trial using feral insects, with the contaminated leaf disc taken directly next to a cadaver disc (spatial structure experiment).

In the half-sibling experiment, the two best-fitting models included effects of full-sib family on detection (Table 2, Fig. 5). The best model also included an effect of sire on cadaver detection, suggesting that the effects of family in our full-sib experiment were in fact due to genetic differences between families, but it was not possible to reject the second best model, for which sire affects overall consumption but not cadaver detection. Our data cannot clearly distinguish between the effects of sire on cadaver detection and the effects of sire on consumption. We therefore suspect that both overall consumption rate and cadaver detection are heritable, especially given that both effects could be detected in our full-sibling experiment.

As we described in the Methods section, producing virus-contaminated foliage required that we place infected larvae

Table 2. Akaike Information Criterion analysis of half-sibling experiments

Model	AIC	Δ AIC	AIC weights
Individual variation	-796.5	212.5	0
+ Presence of a virus disc	-956.0	53.0	0
+ Family's effect on consumption	-970.4	38.6	0
+ Family's effect on consumption + sire's effect on consumption	-968.6	40.4	0
+ Family's effect on detection + sire's effect on consumption	-1008.0	1.0	0.378
+ Family's effect on consumption + sire's effect on detection	-986.4	22.6	0
+ Family's effect on detection + sire's effect on detection	-1009.0	0.0	0.622

**Fig. 5.** Half-sibling trial using feral insects. Each bar represents all of the offspring of a single dam.

on branches in mesh bags in the field for 5 days, but in the first year of our study (conducted in July and August 2006), the control foliage was not held in the bags during this time. For our experiments in that year, this could have induced differences in foliage between the clean and virus-contaminated leaf discs due to mechanisms other than virus contamination. In our second year of experiments (conducted in 2007), however, including roughly half of the total individuals used in the study, we controlled for this effect by placing control foliage in bags alongside our infected bags. As we have already described, the results in the two different years were qualitatively consistent. More quantitatively, if we measure avoidance in terms of the difference in the amount consumed between clean and virus-contaminated discs, then the level of avoidance averaged across individuals was indistinguishable between the 2 years (year 1: mean 0.151 cm², SE 0.010; year 2: mean 0.130 cm², SE 0.010, two-sample *t*-test: $t_{1221} = 1.51$, $P = 0.1324$). It therefore appears that differences in the treatment of control foliage between years had no effect on our results.

Discussion

Our results confirm Capinera *et al.*'s result that gypsy moth larvae can detect and avoid leaves with infected cadavers. Larvae consumed significantly less contaminated foliage

than control foliage in all of our trials. Our data also show that full- and half-sibling feral families differ in the amount of clean foliage consumed and in the extent to which they avoid contaminated leaves. These results suggest that there is a genetic component to the ability to detect virus-contaminated foliage, which is further supported by the observation that the genetically homogeneous laboratory strain did not vary in cadaver detection between families.

Experiments using full-sibling families do not rule out maternal effects, in which differences among egg masses stem from non-genetic attributes of the female parent, but previous work has suggested that such effects are weak in the gypsy moth (Myers, Boettner & Elkinton 1998; Erelli & Elkinton 2000). More directly, the occurrence of sire effects in the model that best described our half-sibling data suggest that cadaver avoidance is heritable, but the data also support the alternative model in which overall consumption is instead heritable. The family effects in our full-sibling trials may thus reflect genetic differences, but clearly more data are needed.

Larvae in our experiments also avoided contaminated foliage even when leaf discs were as much as 0.5 cm away from the cadaver. Spatial structure is known to have an effect on baculovirus transmission (Dwyer 1991; Hails *et al.* 2002; D'Amico *et al.* 2005), and we have demonstrated that the spatial scale at which larvae can detect cadavers is larger than a cadaver. It follows that larvae can avoid the virus even when it is at low concentrations, suggesting that small-scale spatial structure can have large effects on disease transmission. Indeed, Capinera *et al.* (1976) showed that larvae avoid even uninfected cadavers, and as the virus causes the breakdown of the larval integument, larvae that avoid infected cadavers may have been responding to cadaver components rather than to the virus.

Behaviour can thus play an important role in the transmission of insect baculoviruses. Anecdotal observations of larval behaviour in our experiments suggest that larvae consume foliage until they detect cadavers, and then they change position or stop feeding. Indeed, several individual feeding bouts were apparent on many discs, which is in accordance with reports of how gypsy moths feed in the wild (Heinrich 1979; Elkinton & Liebhold 1990). Thus, differences in leaf area consumed between the two discs are probably a reflection of smaller leaf bouts on the virus leaf discs, and differences between families probably result from different tolerances for the cadaver cue. Note that although

vast numbers of different insect species are infected by nucleopolyhedroviruses (NPVs) (Miller 1997), to our knowledge behavioural mechanisms that affect NPV infection risk have been directly studied only in gypsy moths. Nevertheless, Dwyer (1991) provides indirect evidence that movement behaviour affects the risk that Douglas-fir tussock moth larvae (*Orgyia pseudotsugata*) become infected with tussock-moth NPV. Similarly, Hails *et al.* (2002) invoke small-scale spatial structure as a determinant of NPV transmission in the cabbage moth (*Mamestra brassicae*). Moreover, given the intense selection pressure that NPVs impose on many insects (Shepherd *et al.* 1984; Myers 1993; Moreau *et al.* 2005; Moreau & Lucarotti 2007), it seems likely that other insects are also able to detect and avoid cadavers. We therefore suspect that behavioural mechanisms also affect NPV transmission in other insects.

Costs of resistance may explain why this polymorphism exists in gypsy moth populations. Individuals that are more likely to stop feeding in the presence of a cadaver may have reduced fecundity because they may be more likely to stop feeding even when no cadavers are present. Indeed, Capinera *et al.* (1976) showed that larvae also prefer clean leaf discs to molasses-smearred leaf discs, suggesting that larvae may respond to any viscous substance on a leaf, which presumably would have a fitness cost. Moreover, costs of resistance have been observed in many Lepidopteran hosts of baculoviruses (Fuxa & Richter 1998; Lee *et al.* 2006) and other pathogens (Mealor & Boots 2006). Gypsy moth populations in particular undergo dramatic fluctuations in density, and virus-infection rates rise and fall along with density (Woods & Elkinton 1987). This fluctuation in infection rates provides a straightforward mechanism for fluctuations in selection for resistance, which may explain heterogeneity in cadaver-detection ability. Thus, in high-density populations with death due to disease as a strong selective pressure, individuals with behavioural mechanisms for disease avoidance will be favoured. Similarly, in low-density populations with low virus infection rates, natural selection may favour less investment in defence mechanisms.

Indeed, previous work has suggested that variation in infection risk among forest insects can have a strong effect on outbreaks. First, both field transmission experiments and naturally occurring virus epidemics in gypsy moth populations show strong signals of variability in infection risk (Dwyer *et al.* 2000, 2004). More recent work (G. Dwyer, B. D. Elder and M. Coram, unpublished data) has suggested that similar effects also occur in virus epidemics in the Douglas-fir tussock moth (Shepherd *et al.* 1984; Otvos, Cunningham & Friskie 1987), the western tent caterpillar (Myers 2000) and the spruce sawfly (Moreau & Lucarotti 2007). As our work has shown that heterogeneity in infection risk may be due to heterogeneity in behaviour, it suggests that behaviour can modulate the effects of host density on epidemic severity. Moreover, as the effects of behaviour are seen at small spatial scales, and because our work has shown that small-scale spatial structure can also affect transmission, we suspect that

behaviour and spatial structure may interact to determine the effects of density on epidemic severity. Second, our data suggest that variability in behaviour is heritable, and in insect–pathogen models, realistic levels of variability in infection risk produce stability unless the variability is heritable (Elder *et al.* 2008). By providing evidence that variability in cadaver avoidance behaviour is heritable we have identified a mechanism that may allow realistic cycles in insect–pathogen models, thus allowing the models to be connected to the biology of insect–NPV interactions in nature.

More generally, our work emphasizes the important role of behaviour in determining host resistance. Although variability in behaviour is widely acknowledged to play a key role in the spread of diseases of humans and other vertebrates (Anderson & May 1992; Kiesecker & Skelly 2000), studies of invertebrate pathogens generally focus on variability in innate susceptibility (Miller 1997). Our work, by contrast, suggests that variability in behaviour may play a key role in insect resistance to baculoviruses, adding to the limited body of research in this area. We therefore argue that variability in behaviour is a neglected issue in studies of variability in disease resistance in invertebrate hosts.

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Supporting Information

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Table S1. Akaike Information Criterion analysis of infection rate data.

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