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The infection rate of *Daphnia magna* by *Pasteuria ramosa* conforms with the mass-action principle

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SUMMARY

In simple epidemiological models that describe the interaction between hosts with their parasites, the infection process is commonly assumed to be governed by the *law of mass action*, i.e. it is assumed that the infection rate depends linearly on the densities of the host and the parasite. The mass-action assumption, however, can be problematic if certain aspects of the host–parasite interaction are very pronounced, such as spatial compartmentalization, host immunity which may protect from infection with low doses, or host heterogeneity with regard to susceptibility to infection. As deviations from a mass-action infection rate have consequences for the dynamics of the host–parasite system, it is important to test for the appropriateness of the mass-action assumption in a given host–parasite system. In this paper, we examine the relationship between the infection rate and the parasite inoculum for the water flee *Daphnia magna* and its bacterial parasite *Pasteuria ramosa*. We measured the fraction of infected hosts after exposure to 14 different doses of the parasite. We find that the observed relationship between the fraction of infected hosts and the parasite dose is largely consistent with an infection process governed by the mass-action principle. However, we have evidence for a subtle but significant deviation from a simple mass-action infection model, which can be explained either by some antagonistic effects of the parasite spores during the infection process, or by heterogeneity in the hosts' susceptibility with regard to infection.

INTRODUCTION

The rate of infection is commonly assumed to depend linearly on the density of the host and the parasite, an assumption which stems from the theory of the kinetics of chemical reactions where it is referred to as the *mass-action principle*. The mass-action principle was introduced into epidemiology by Hamer [1]. Many aspects of the interaction between hosts and parasites, however, may render a mass-action infection term inappropriate for a realistic description of the infection process. Firstly, one has to carefully consider whether the details of the transmission of a given parasite are consistent with the mass-action principle. In general, one cannot expect the infection term to be linear in the densities of parasites and hosts [2]. The transmission of cowpox virus in rodents, for example, was recently found to be best described by an infection term that is linear in the frequency, rather than the density, of infected individuals [3]. Secondly, a mass-action infection term implicitly assumes a homogeneously mixed system. Consequently, if the host–parasite system in question is structured spatially, the term that best describes the infection process may not conform to the mass-action principle [4, 5]. Another important aspect that is often neglected in

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epidemiological models is the host's immune system. An immune system may enable the host to clear the parasite before it can cause harm, reproduce, or transmit [6, 7]. If immunity depends on the density of infectious particles then it will influence the dependence of the infection term on the parasites density. Finally, if the host individuals differ with respect to their susceptibility for the parasite, the infection term will not be linear in the densities of hosts, and will, therefore, also deviate from a mass-action infection term.

The form of the infection term has important consequences for the dynamical behaviour of the host–parasite system. An infection rate which increases over-proportionately with the parasite dose results in an invasion threshold for the parasite [7–15]. If the infection rate increases under-proportionately with the parasite dose, as is the case if host heterogeneity plays an important role, parasite invasion is facilitated [16, 17].

Here, we investigate how the rate of infection of the water flea *Daphnia magna* depends on the density of its parasite *Pasteuria ramosa* to which it is exposed. The main question we try to answer is how well a model with a mass-action infection term describes this particular host parasite system. In the next section we describe the experiment that gave rise to data on the fraction of infected hosts for 14 different parasite densities. In Section 3, we study mathematical models with different infection terms, and derive expressions for the fraction of infected hosts as a function of the parasite dose. The derived expressions are fitted to the data of our experiment in Section 5. The main conclusion of our analysis is that a model assuming the interaction of homogeneous host and parasite populations according to a mass-action infection term is in good agreement with the data. Nevertheless, we can identify a subtle, but highly significant deviation from this model. The deviation can be explained either by antagonism of parasite spores during the infection process, or by heterogeneity of the host population with regard to susceptibility to infection.

MATERIAL AND METHODS

Host and parasite

Daphnia magna Straus is a cyclically parthenogenetic zooplanktor common to many small to medium size water bodies in Eurasia. Newborn length is about 0·7–0·9 mm and adult length between 2 and 5 mm. Sexual reproduction is triggered by poor environmental conditions, but in this study only asexual reproduction was studied as experimental conditions did not permit the hatching of sexually produced resting eggs. *D. magna* were collected in September 1997 from a pond close to Gaarzerfeld in North Germany. From this sample an isofemale line (i.e. clone) was produced by isolating parthenogenetic eggs from the brood chamber of an adult female and raising the clonal offspring in isolation. Isolating eggs avoided possible transmission of water-borne parasites from the mother to the offspring. We used only one clone (DG-1-106) for this study.

Pasteuria ramosa is a bacterial parasite that infects the host's body cavity [18]. Hosts become castrated shortly after infection. Infective spores are set free from decaying host bodies, hence, killing the host is necessary for transmission. Transmission takes place through contact between the host and the water-borne spores. It is not clear currently whether the parasites enters the host through penetration of the carapace or through ingestion by the filter feeding host. This parasite is not vertically transmitted. The isolate used in this study was collected from the same population at the same time as the host clone was isolated. To obtain spores of this parasite, we took one naturally infected female and kept her in good food condition until she died from the infection. We collected the spores of the parasite and infected offspring of host clone DG-1-106. One infected host was used to propagate the spores and then to infected again offspring of DG-1-106. This bottleneck procedure was repeated three times, before we infected about 100 offspring of DG-1-106. These 100 offspring were well fed to produce many parasite spores, which we needed for the following experiment. An infected *D. magna* may produce up to 80 million spores of *P. ramosa* (on average about 20–30 million spores). The infected females were frozen at $-20\,^{\circ}$C, around the time they would die from the disease (40–50 days post-infection).

The spore solution used in the experiment was prepared by taking 62 frozen, infected, female *D. magna*, keeping them at room temperature for 30 min and then grinding up their tissue to release the parasite spores. The solution was filtered (mesh width 25 μm) and the spore concentration quantified with a haemocytometer. It was diluted to 82 ml with 13·3 million spores per ml. This gave rise to a suspension of 6 million spores per 20 ml. Suspensions of lower spore concentrations were prepared by threefold dilutions.

The lowest concentration was expected to contain 11·3 spores per 20 ml.

General experimental conditions

Throughout the experiments, artificial culture medium [19], modified as described in [20] was used. All *Daphnia* cultures and experiments were fed with the unicellular algae *Scenedesmus gracilis* cultured in chemostats. All experiments except the microcosm experiment were run under constant light/dark conditions (16:8) with a water temperature of $20 \pm 0{\cdot}5$ °C in incubators. All treatments were equally distributed across the two incubators used for the experiment.

The experiment

An experiment was carried out with 112 independent replicates per spore dose (13 doses plus control = 1568 jars).

To start the experiment, we used second brood neonates derived from 600 *Daphnia* females of a single clone (DG-1-106) and kept in groups of 20 individuals per liter under good food conditions ($2{\cdot}5 \times 10^5$ algae cell per ml medium per day). From the first clutch of the 600 *Daphnia*, 1568 newborns were collected and randomly assigned to 14 treatment groups. Each of the 1568 individuals was kept individually in 20 ml for 4 days. During this time the *Daphnia* were exposed to the parasite by adding spore suspension into the culture medium. Animals were fed daily with 10^5 algae cell per ml. After 4 days all *Daphnia* were individually placed in 100 ml fresh medium. Subsequently medium was replaced every 7 days. At day 12, 16 and 20 we increased the daily food level to 3×10^4, 4×10^4 and 5×10^4 algae cells per ml medium, respectively, to accommodate the food demands of the growing *Daphnia*. Dead animals were recorded daily. Animals, which died after day 22 were dissected and checked for disease. Animals which died earlier could not reliably checked for infections and were therefore not included into the analysis.

At day 30 post-infection all animals were scored for infection. Infections are clearly visible because infected animals have a brownish-reddish colour and do not carry eggs. In cases of doubt, animals were dissected and checked under 400× magnification. However, in all cases were we had doubt, the microscopic investigation confirmed our initial diagnosis. Animals scored uninfected at day 30 were again checked at day 44 to verify their status. However, no more infected animals were found.

WHAT RELATION BETWEEN THE FRACTION OF INFECTED HOSTS ANDS THE PARASITE DOSE DO WE EXPECT?

In this section, we introduce a mathematical model that describes our experiment, and derive expressions for the expected relation between the fraction of infected hosts and the parasite dose to which the hosts are exposed.

The following population dynamical model describes the interaction between the parasite, P, the uninfected hosts, H, and infected hosts, I, during the experiment:

$$\dot{P} = -cP \tag{1}$$

$$\dot{H} = -\beta(P)H \tag{2}$$

$$\dot{I} = \beta(P)H \tag{3}$$

Here, $\beta(P)$ is the infection rate of a certain parasite concentration, P, per host individual. The initial values for our experiment are $H(0) = H_0$, $I(0) = 0$ and $P(0) = P_0$. P_0 is the dose to which the hosts are exposed. The model ignores host reproduction and mortality. Thus the total number of hosts does not change during the experiment, i.e. $H(t) + I(t) = H_0$.

This model is simple enough to allow an analytical solution of its dynamics. If we ignore the death rate of the parasite, i.e. $c = 0$, which is justified for the 4 days of the experiment as spores can survive several decades (D. Ebert, unpublished observations), the solutions read:

$$P(t) = P_0 \tag{4}$$

$$H(t) = H_0 \mathrm{e}^{-\beta(P_0)t} \tag{5}$$

$$I(t) = H_0(1 - \mathrm{e}^{-\beta(P_0)t}) = H_0 - H(t) \tag{6}$$

In the experiment, we measure the fraction, f, of infected hosts after a certain time, t_{exp}, has elapsed. f can be defined in term of the variables H and I as

$$f := \frac{I(t_{\mathrm{exp}})}{I(t_{\mathrm{exp}}) + H(t_{\mathrm{exp}})} = \frac{I(t_{\mathrm{exp}})}{H_0} \tag{7}$$

The fraction of infected hosts, f, varies with P_0, the initial parasite dose according to the following equation:

$$f = 1 - \mathrm{e}^{-\beta(P_0)t_{\mathrm{exp}}} \tag{8}$$

The form of f as a function of the parasite inoculum P_0 depends on the function $\beta(P_0)$. Figure 1*a* shows

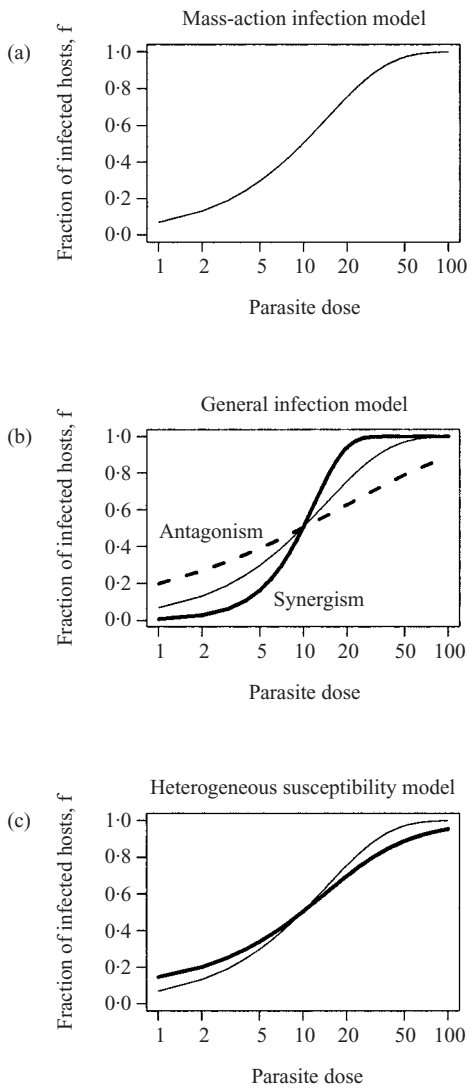

Fig. 1. Theoretical expectations for the fraction of infected hosts, f, as a function of the parasite inoculum, P_0: (a) the mass-action infection model (equation 10), (b) the general infection model (equation 12) that incorporates synergistic/antagonistic interaction between the parasites during the infection process, and (c) the heterogeneous susceptibility model (equation 13) that assumes normally distributed host susceptibilities. The thin line in (b) and (c) shows the mass-action infection model (equation 10) for comparison.

f as a function of P_0 for a mass-action infection term, $\beta(P_0)=bP_0$. For a mass-action infection term, the fraction of infected hosts, f, is a saturating function of the parasite dose, saturating at 1. (This also holds if we take parasite mortality into account. In that case f converges to 1 more slowly.)

For an infection term that deviates from a mass-action infection term, the fraction of infected hosts, f, will differ in mainly two regards: first, if the infection term is bounded from above, i.e. there is a maximum infection rate, f may saturate at a lower level than 1, and second, the pattern of increase to the saturation level may differ.

In this paper we consider two potential infection terms: (i) a common mass-action infection term, and (ii) a term that describes synergism or antagonism of parasite spores during the infection process. Moreover, we consider a model which takes into consideration that the host individuals may differ with respect to their susceptibility to infection.

Mass-action infection terms

To determine how well a mass-action infection term describes our data, we consider a mass-action infection term:

$$\beta(P_0)=bP_0 \tag{9}$$

This infection term leads to the following relation between the fraction of infected hosts and the parasite dose to which the hosts are exposed:

$$f=1-\mathrm{e}^{-bP_0t_{\exp}} \tag{10}$$

We will refer to this model as the *mass-action infection model*. Fitting the mass-action model (equation 10) to the data of our experiments will give us an estimate for the infection rate, b.

Equation 10 for the fraction of infected hosts, f, also holds if the parasite population is heterogeneous with regard to the infectivity of individual parasite spores (as shown in Appendix A), or if stochastic effects are taken into account (as shown in Appendix B). In the case of heterogenous parasite infectivities, the parameter b should be interpreted as the average infection rate.

Synergism or antagonism of parasite spores

To determine whether there is evidence for a systematic deviation from a mass-action infection model (equation 10) in our data, we consider the following general infection term:

$$\beta(P_0)=\tilde{b}P_0^{\kappa} \tag{11}$$

For $\kappa=1$ this infection term is simply the mass-action infection term. For $\kappa>1$ an increase in the parasite inoculum will lead to an over-proportionate increase in the infection rate per host, and thus describes a synergistic effect of the parasite spores during the infection of hosts. For $\kappa<1$ an increase in the parasite inoculum will lead to an under-proportionate increase

in the infection rate per host, and thus describes an antagonistic effect of the parasite spores. The function $\tilde{b}P_0^{\kappa}$ is not bounded from above, i.e. $\beta(P_0)$ increases indefinitely with increasing parasite inoculum, P_0, and thus $f=1-\mathrm{e}^{-\beta(P_0)t_{\exp}}$ will converge to 1. To exclude infection terms which are bounded from above is justified since our data show that the fraction of infected hosts, f is 1 for very high inocula. Thus, even though the "real" infection term may have a maximum, our data suggest that this maximum is very high – too high to have played a significant role in our experiment.

Fitting the *general infection model*

$$f=1-\mathrm{e}^{-\tilde{b}P_0^{\kappa}t_{\exp}} \tag{12}$$

to the data of our experiment will give us estimates of the parameters $\tilde{b}$ and κ and their standard errors. If our estimate for κ is significantly different from 1, we have evidence that the infection process cannot be adequately described by the mass-action infection model (equation 10). The sign of $\kappa-1$ will further inform us about potential synergism or antagonism of parasite spores during the infection of a host individual. For a comparison between the general infection model (equation 12) and the mass-action infection model (equation 10), see Figure 1*b*.

Host heterogeneity in susceptibility to infection

The host population could consist of host individuals that differ in their susceptibility to infection. In our experiment, such differences in susceptibilities could arise, for example, from differences in age. (The *Daphnia* were born within 48 h which could produce a minor age effect.) Let us assume that, for a given host individual, the relation between the parasite dose and the infection rate per host is governed by the mass-action principle. Different host individuals, however, are characterized by different infection rates. We can easily incorporate heterogeneous susceptibilities by assuming a distribution of host susceptibilities, $D(b)$. We can calculate the fraction of infected hosts as

$$f=1-\int_0^{\infty} D(b)\mathrm{e}^{-bP_0t_{\exp}}\,\mathrm{d}b \tag{13}$$

We will refer to this model as the *heterogeneous susceptibility model*.

We do not know too much about the distribution of the infectivities, b, and the real distribution could be very complex. For simplicity, we will assume in the following that b is normally distributed. Our results, however, do not qualitatively depend on this assumption. Fitting the heterogeneous susceptibility model (equation 13) to our data will result in estimates of the mean and the standard deviation of the distribution of infectivities.

Figure 1*c* shows that the heterogeneous susceptibility model (equation 13) predicts a more moderate increase of the fraction of infected hosts, f, than the mass-action infection model (equation 10).

It is important to note that, as the mass-action infection model (equation 10), also the heterogeneous susceptibility model (equation 13) conforms with the mass-action principle, i.e. the probability of infection for a given hosts individual is still proportional to the parasite concentration. The main difference between the model is that the heterogeneous susceptibility model takes into account that the host population may not be homogeneous. The fact that the heterogeneous susceptibility model (equation 13) differs from the mass-action infection model (equation 10) illustrates how the population structure may conceal the principles that govern the interaction between species in ecological systems.

RESULTS

We exposed individuals of the host *Daphnia magna Straus* to 14 different doses of the bacterial parasite *Pasteuria ramosa* for 4 days and recorded if this exposure resulted in an infection. For each dose the experiment was repeated for 112 host individuals, which allowed us to determine the fraction of infected hosts with an error of 1%. The results of the experiment are summarized in Table 1. In all treatment groups (including the control group) some host individuals died for unknown reasons. Therefore, the total numbers of hosts in Table 1 are smaller than 112.

We fitted the mass-action infection model (equation 10), the general infection model (equation 12) and the heterogeneous susceptibility model (equation 13) to our data using the least-squares algorithm `nls()` of the R language for statistical computing [21]. We fitted the arcsin-square-root-transformed fractions of infected hosts to normalize the distribution of our data.

Fitting the mass-action model (equation 10), we obtain the following estimate for the infection rate, b:

$$b=2{\cdot}63\times10^{-4}\,\mathrm{ml/day},\quad \mathrm{SE}_b=0{\cdot}40\times10^{-4}\,\mathrm{ml/day} \tag{14}$$

(Here and in the following, the standard errors of the estimates are based on a local quadratic approximation to the non-linear least-squares predictor.) For

Table 1. *The number of infected and uninfected hosts after a 4-day exposure to different parasite doses. To calculate the corresponding host and parasite concentrations we divided the above numbers by the volume of water in the jars (20 ml)*

Dose	Infected hosts	Uninfected hosts	Total hosts
$6{\cdot}00\times10^{6}$	102	0	102
$2{\cdot}00\times10^{6}$	93	1	94
$6{\cdot}67\times10^{5}$	76	0	76
$2{\cdot}22\times10^{5}$	96	1	97
$7{\cdot}41\times10^{4}$	95	10	105
$2{\cdot}47\times10^{4}$	77	28	105
$8{\cdot}23\times10^{3}$	37	66	103
$2{\cdot}74\times10^{3}$	26	76	102
$9{\cdot}14\times10^{2}$	10	97	107
$3{\cdot}04\times10^{2}$	7	93	100
$1{\cdot}02\times10^{2}$	0	105	105
$3{\cdot}39\times10^{1}$	0	102	102
$1{\cdot}13\times10^{1}$	1	106	107
0·00	0	96	96

a given the infection rate, b, the infectious dose, ID_{50}, at which 50% of the hosts become infected can be calculated as $\mathrm{ID}_{50}=\ln 2/(t_{\mathrm{exp}}b)$. For our estimate of the infection rate, b, we obtain

$$\mathrm{ID}_{50}=659\,\mathrm{spores/ml} \tag{15}$$

Note that the above estimate for the ID_{50} depends sensitively on the time of exposure of hosts to parasites, t_{exp}.

Figure 2*a* shows that our data are well described by the mass-action infection model (equation 10). A close look, however, reveals that there is a systematic deviation in the data from the mass-action model: the fitted curve is 'steeper' than the observed fraction of infected hosts.

Fitting the general infection model (equation 12) to the data improves the fit significantly ($p=0{\cdot}0041$, F-test). We obtain the following estimates for b and κ:

$$\tilde{b}=1{\cdot}47\times10^{-3}\,\mathrm{ml}^{\kappa}/\mathrm{day},\quad \mathrm{SE}_{\tilde{b}}=0{\cdot}60\times10^{-3}\,\mathrm{ml}^{\kappa}/\mathrm{day}$$

$$\kappa=0{\cdot}739,\qquad \mathrm{SE}_{\kappa}=0{\cdot}059 \tag{16}$$

The fact that the estimate for κ is significantly smaller than one, suggests that an increase in the parasite concentration does not give rise to a proportionate increase of the infection rate. For the present estimate for κ a tenfold increase in the parasite concentration results in only a fivefold increase of the infection rate per host.

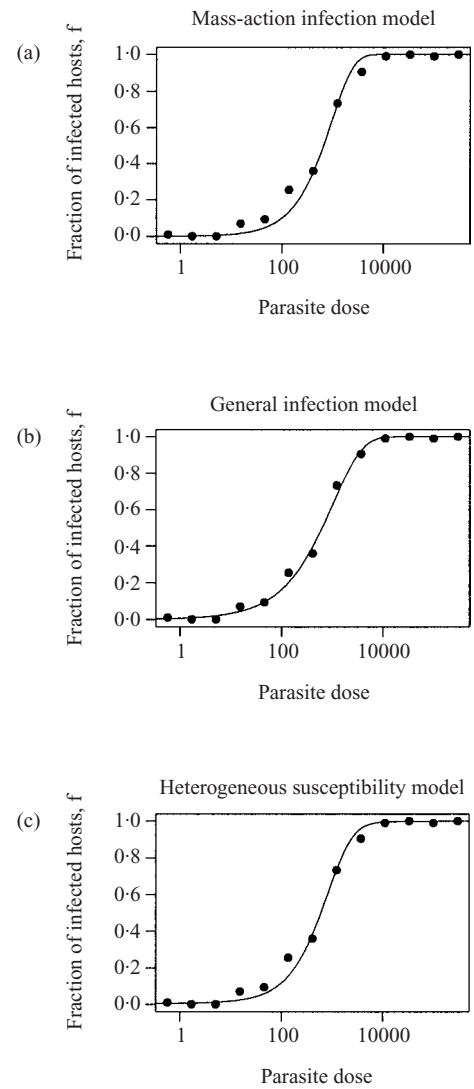

Fig. 2. Fitting (a) the mass-action infection model (equation 10), (b) the general infection model (equation 12), and (c) the heterogeneous susceptibility model (equation 13) to our dataset. The mass-action infection model (equation 10) is largely consistent with the data, but overestimates the slope of f at intermediate parasite doses. The general infection model (equation 12) and the heterogeneous susceptibility model (equation 13) fit significantly better (F-test on the residual sum of squares: $p=0{\cdot}00036$ and $p=0{\cdot}021$, respectively).

In Figure 3, we compare the infection term of the mass-action infection model (equation 10) and the general infection model (equation 12). For low parasite doses, the general infection model (equation 12) predicts higher rates of infection, and for high parasite doses lower rates of infection than the mass-action infection model (equation 12), which is, in essence, the reason for the better fit of the general infection model. The two infection terms, bP_0 and

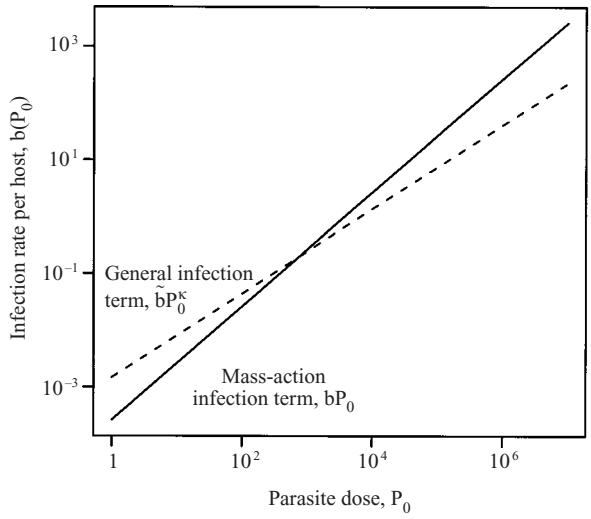

Fig. 3. Comparing the infection terms of the fitted mass-action infection model (equation 10), bP_0, (solid line) and the fitted general infection model (equation 12), $\tilde{b}P_0^{\kappa}$ (dashed line). For low parasite doses, the general infection model (equation 12) predicts higher rates of infection, and for high parasite doses lower rates of infection than the mass-action infection model (equation 10). The infection terms coincide at approximately the ID_{50}.

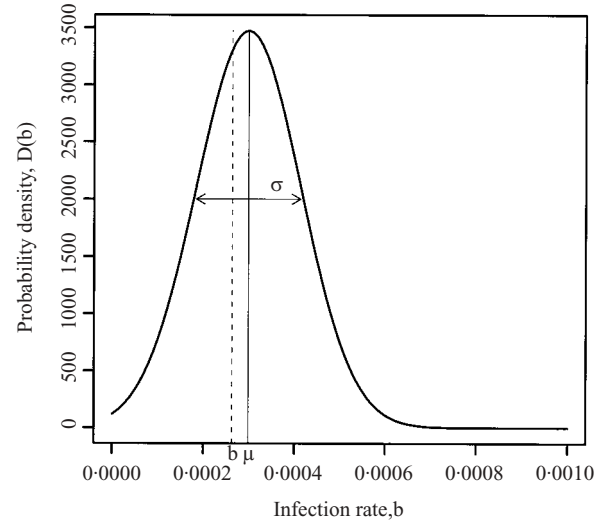

Fig. 4. Estimated distribution of host susceptibilities. The estimate of the infection rate b of the mass-action infection model (equation 10) is also plotted for comparison.

$\tilde{b}P_0^{\kappa}$, coincide at intermediate parasite doses (see Fig. 3).

The deviation of the data from the prediction of the mass-action infection model (equation 10) can also be explained by assuming that the host population is heterogeneous with regard to susceptibility. Fitting the heterogeneous susceptibility model gives a significantly better fit than the mass-action infection model (equation 10) ($p=0{\cdot}038$, F-test). The estimates for the mean, μ, and the standard deviation, σ of the normally distributed infectivities, b, are given by

$$\mu = 2{\cdot}99 \times 10^{-4}\,\text{ml/day}, \quad \text{SE}_{\mu} = 0{\cdot}43 \times 10^{-4}\,\text{ml/day} \tag{17}$$

$$\sigma = 1{\cdot}15 \times 10^{-4}\,\text{ml/day}, \quad \text{SE}_{\sigma} = 0{\cdot}25 \times 10^{-4}\,\text{ml/day} \tag{18}$$

The estimated mean of the distribution of susceptibilities, μ, and the estimated parameter b of the mass-action infection model (equation 10) are not statistically different ($p>0{\cdot}25$, t-test). Moreover, the standard deviation of the fitted distribution is significantly different from zero ($p=0{\cdot}0007$, t-test), i.e. the estimated distribution characterizes genuinely heterogeneous susceptibilities. Thus, the fact that the heterogeneous susceptibility model (equation 13) fits significantly better than the mass-action infection model (equation 10) is due to the non-vanishing variance of the susceptibilities in the heterogeneous susceptibility model (equation 13). Figure 4 shows the fitted distribution of infectivities.

Intuitively, the heterogeneous susceptibility model (equation 13) gives a better fit because, at low parasite doses, highly susceptible hosts are abundant, thus leading to disproportionately more infections at low parasite doses, whereas at high parasite doses, the pool of susceptible hosts is exhausted early, and the remaining susceptible hosts are more difficult to infect. This gives rise to a slower increase of the fraction of infected hosts f in the heterogeneous susceptibility model (equation 13) than in the mass-action infection model (equation 10).

DISCUSSION

We performed an experiment determining the dependence of the infection rate of *Daphnia magna* on the dose of the bacterial parasite *Pasteuria ramosa*. We analysed the dataset using specifically designed mathematical models. We showed that the infection process in our experiment is satisfactorily described by the model based on the mass-action assumption which is commonly adopted by models describing the interaction between parasites and their hosts. However, our data display a subtle, but significant systematic deviation from the prediction of the mass-action infection model: the increase in the fraction of infected hosts as a function of the parasite dose is slower than expected.

The deviant pattern is consistent with two potential mechanisms. Firstly, parasite spores could act antagonistically during the infection process. As a consequence, the infection term would be non-linear such that the infection rate increases less than linearly with the parasite dose. We constructed a simple mathematical model that formalizes potential antagonism (or synergism) between the parasite spores during infection. Our data are best compatible with an infection term according to which an increase in the parasite dose by a factor 10, leads to an only fivefold increase in the infection rate. The resulting fit is significantly better than the fit of the mass-action infection model (equation 10).

The second explanation of the deviation of the prediction of the mass-action infection model from the experimental data does not involve alternative (i.e. non-mass-action) infection terms, but invokes heterogeneity with regard to the hosts' susceptibility to parasite infection. If we assume that the host population, though clonal, is composed of phenotypically differently susceptible host individuals, we can also account for the slower increase of the fraction of infected hosts with the parasite dose. A simple model assuming normally distributed host susceptibilities, fits the data significantly better than the mass-action infection model (equation 10). Intuitively, in a scenario with a heterogeneous host population, the more susceptible hosts would be infected first, thus accounting for the observation that the fraction of infected hosts at low parasite doses is higher than predicted by the mass-action infection model (see Fig. 2*a*). At high doses, the parasites face the challenge of having to infect hosts which are less susceptible, which explains why the fraction of infected hosts is lower than the prediction of the mass-action infection model.

These two hypotheses are probably not the only ones that can explain the observed deviation from the mass-action infection model. On the basis of our analysis, we cannot decide whether parasite antagonism, heterogeneous susceptibilities, or yet another factor is the reason of the deviation of our observations from the prediction of the mass-action infection model. Yet, we can exclude certain factors from the list of possible explanation of the deviation. Firstly, theoretical analysis shows that parasite heterogeneity with regard to infectivity is not able to account for the deviation. A system with a heterogeneous parasite population is not expected to deviate systematically from the prediction of the mass-action infection model (see Appendix A). Secondly, stochastic effects can also not explain the deviation between the observed fraction of infected hosts and the predictions of the mass-action model (see Appendix B). Furthermore, we tried to rule out strong spatial effects in our experiment by design. We minimized the possibility of spatial variation in host and parasite concentrations by keeping each host individual in a separate jar of defined volume, and by challenging each individual host with a well mixed solution of parasite spores. Lastly, host immunity is also unlikely to offer an explanation for the deviation between our observations and the mass-action infection model. Taking into account host immunity will lead to a relative disadvantage of low dose challenges, and is thus expected to result in a deviation that goes into the opposite direction: the observed fraction of infected hosts should increase faster, not slower than the mass-action infection model predicts.

In future experiments, one could address the question of the mechanism behind the deviation from the mass-action infection model. The potential antagonism between parasite spores is difficult to study unless the mechanism of antagonism is revealed. To study the role of heterogeneity in host susceptibility, one could conduct an experiment with different degrees of host heterogeneities. To that end, it may be useful to identify correlates of susceptibility, such as, for example age or size, which would allow to easily control for different degrees of heterogeneity in susceptibility. In such an experiment, the deviation from the prediction of the mass-action infection model is expected to increase with increasing degrees of host heterogeneity.

Deviations from the mass-action principle have important dynamical consequences. We and others have shown that, if the infection rate that increases *over*-proportionately with parasite concentration at low doses, parasite invasion is aggravated [7–15]. By extrapolation, the finding of our present study, i.e. that the infection rate per host effectively increases *under*-proportionately with parasite concentration will result in facilitated parasite invasion. In the case in which the effective under-proportionate increase of the infection rate can be attributed to host heterogeneity, it has been shown for specific sexually transmitted diseases [22, 23], as well as in a general context [16, 17] that the basic reproduction ratio – a measure of the parasite's ability to invade – is larger in a heterogeneous host population than in a

homogeneous system with equal average susceptibility. Therefore, regardless of the cause that underlies the deviation from the mass-action principle, our finding that the infection rater per host effectively increases under-proportionately with parasite concentration will result in facilitated parasite invasion. Thus, while the mass-action assumption may be a good approximation for an equilibrated system, deviations from the mass-action principle may become more relevant when investigating the invasion of parasites into an uninfected host population.

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APPENDIX A: DIFFERENTIAL PARASITE INFECTIVITIES

Here, we show that taking into consideration the potential variation in parasite infectivity leads to a relation between the parasite dose and the fraction of infected hosts, f, that is equivalent to the mass-action infection model (equation 10).

Let us assume that the parasite strains differ with regard to their infectivities, b. Let $D(b)$ be the distribution of infectivities in the parasite population. Assuming that parasite mortality is negligible (as in equation 4) we can derive the following expression for the fraction of infected hosts:

$$f = 1 - \mathrm{e}^{-\left(\int_0^\infty bD(b)\,\mathrm{d}b\right) P_0 t_{\exp}} \tag{A1}$$

$$= 1 - \mathrm{e}^{-\bar{b} P_0 t_{\exp}} \tag{A2}$$

Hereby, $\bar{b}$ is the average infectivity of the parasite population.

Note that the relation between the fraction of infected hosts and the parasite dose depends only on the average infectivity of the parasite population, and not on higher moments of the distribution such as the variance of infectivities. Thus equation A2 is equivalent to the mass-action infection model (equation 10).

APPENDIX B: STOCHASTIC MODEL

Here, we show that taking into consideration stochastic effects leads to a relation between the parasite dose and the fraction of infected hosts, f, that is equivalent to the mass-action infection model (equation 10).

Let us construct a stochastic version of the simple model (equations 1–3). Let n be the number of host individuals that were exposed to a certain dose level in our experiment. For example, for the highest dose the number of challenged hosts was $n = 102$. A stochastic system with n host individuals can attain $n+1$ states: the state in which none of the host individual is infected, the state in which only one host individual is infected, etc. Let r denote the state in which we have r uninfected host individuals, and let p_r be the probability that the system is in state r. If we assume that the concentration of parasites remains constant during the experiment (as we assume in the deterministic model (equation 10)), the stochastic model is given by the following equations that specifies the time progression of the probabilities p_r:

$$\dot{p}_r = bP_0(r+1)p_{r+1} - bP_0 r p_r, \quad r = 0, \ldots, n-1 \tag{B1}$$

$$\dot{p}_n = -bP_0 n p_n \tag{B2}$$

The initial condition is $p_n = 1$, i.e. the system is with certainty in the state in which all hosts are uninfected.

To compare the stochastic with the deterministic model we calculate the average number of uninfected hosts at time $t_{\exp}$:

$$m(t_{\exp}) = \sum_{r=0}^{n} r p_r(t_{\exp}) \tag{B$\pm$3}$$

Summing over equations B1–B2 we get

$$\dot{m}(t_{\exp}) = -bP_0 m(t_{\exp}) \tag{B4}$$

which has the solution

$$m(t_{\exp}) = n\mathrm{e}^{-bP_0 t_{\exp}} \tag{B5}$$

This expression allows the calculation of the fraction of infected hosts in the stochastic model, f:

$$f = \frac{n - m(t_{\exp})}{n} = 1 - \mathrm{e}^{-bP_0 t_{\exp}} \tag{B6}$$

which is equal to the fraction of infected hosts in the deterministic model. The equality of the predictions of the mass-action infection model and the stochastic model is due to the fact that the terms in the models are linear.

Thus, stochastic effects are not expected to have any systematic impact on the fraction of infected hosts.

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