Does multiple infection select for raised virulence?

Sam P. Brown, Michael E. Hochberg and Bryan T. Grenfell

Classical models of virulence evolution conclude that the increased competition favoured by multiple infection will select for increasing consumption and deterioration of the host resource, or 'virulence'. However, recent empirical and theoretical studies suggest that this view of virulence has some shortcomings. Here, we argue that the evolutionary consequences of multiple infection depend critically on whether the exploitation rate of an individual parasite is governed directly by the behaviour of the individual, or whether it is limited by the collective behaviour of the coinfecting group. We illustrate that, depending on the mechanistic details of exploitation, multiple infection can select for reduced virulence.

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Recent models of the evolution of VIRULENCE (see Glossary) have focused on two associated levels of parasite adaptation [1–4]. The first involves an optimization problem – how to balance reductions in the quality and quantity of resources owing to host exploitation against the benefits of rapid growth, multiplication and transmission (this mirrors the classic trade-off between fecundity and longevity). Extending this framework to multiple infection introduces the second important ingredient in evolutionary models of virulence, the problem of competition among parasites. The generic problem of competing for a shared limited resource is often referred to as the TRAGEDY OF THE COMMONS. Specifically, the problem for a parasite following a strategy of prudent exploitation, as determined by the trade-off between fecundity and longevity, is that should a competing parasite in the same host grow and/or reproduce more quickly, then the quality and quantity of resources available from the host will decline rapidly, penalizing the prudent strain.

One of the basic tenets of modern models of virulence evolution is that when hosts are infected with multiple genotypes (reducing within-host RELATEDNESS), virulence is raised owing to the operation of the tragedy of the commons [1–4]. In a comprehensive review of evolutionary models of virulence, Frank [2] states categorically that 'higher virulence is favoured by lower relatedness in all cases, both for endemic and epidemic infections, and for trade-offs with either transmission or clearance'. Despite the ubiquity of this theoretical conjecture, empirical tests have been limited by the basic problem of evolutionary experiments – the slow passage of evolutionary time. Inevitably, the one notable empirical success was in a viral system. Turner and Chao [5] were able to investigate the role of within-host relatedness in the evolution of virulence (measured as the exploitation rate of a non-evolving bacterial host) by specifying different ratios of infecting viral particles to bacterial host cells. In contrast to the prediction cited above, they found that viral lineages experiencing consistently low within-host relatedness displayed reduced rates of host exploitation, or virulence.

'To interpret the impact of multiple infection on the evolution of virulence in a particular system, it is…essential to understand whether the reproductive or exploitative rate of an individual parasite depends directly on the behaviour of the individual, or whether it is limited by the collective action of the coinfecting group.'

Turner and Chao [5] interpreted their results in terms of viral cooperation and defection, with cooperation entailing the manufacture of the shared intracellular products required for phage reproduction [5–7]. Thus, under conditions of high relatedness, cooperative and collective host exploitation is favoured. In this article we highlight how parasite exploitation traits are often expressed on a collective or group level (for example, the rate of phage exploitation of a bacterial host is defined by the total group production of shared intracellular products), indicating that the flip-side of the tragedy of the commons – COLLECTIVE ACTION – is a common force in nature (Fig. 1). As in 'tragedy' models, 'collective' models predict that decreases in within-host relatedness lead to a shift from group (or single-genome) optimization to a state of individual self-interest and social 'cheats'. However, the two models represent different temptations to cheat. Traits embodying the tragedy of the commons present the temptation of rapaciousness, or taking too much, as the rate of exploitation is limited by the behaviour of the individual and costs are met by the group. By contrast, collective action traits present the temptation of 'free-loading', or giving too little, as the rate of exploitation is limited by the collective action of the group, with costs being met by direct individual contributions to the common good [8–12] (Fig. 1).

Individual versus collective mechanisms of exploitation

In Box 1 we introduce a simple model to illustrate the contrasting evolutionary consequences of 'tragedy'

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Glossary

Collective action: concerns the provision of 'public goods'. The construction of public goods – goods available to all – poses the problem of 'free-riders', selfish individuals who gain by diminishing their contribution to the public good. The notion of collective action has a long history in economics [a] but has only recently attracted attention in biology [b].

Evolutionarily stable: evolutionarily stable strategies (ESS) are phenotypes (i.e. behaviours or morphologies) that, when adopted by all members of a population, ensure that any rare deviant has lower fitness than the resident type following the ESS. The ESS represents an important application of game theory to biology [c,d].

Quorum sensing: by monitoring the extracellular density of a constitutively produced signal molecule, individual quorum-sensing bacteria can limit their expression of group-beneficial phenotypes (e.g. secretion of virulence factors or antibiotics or biofilm construction) to cell densities that are likely to favour an effective group outcome [e]. Bacterial pathogens typically produce a wide range of quorum-regulated exoproducts, many of which contribute to virulence through their effects on their host [f].

Relatedness: a measure of genetic similarity between individuals, relative to the population mean. Increasing the multiplicity of infection reduces relatedness among parasites sharing a host. The degree of competition among parasite individuals within a host can be modulated using a coefficient of relatedness, *r*, with low relatedness favouring increased competition, even if the intensity of infection remains constant [d,g,h].

Tragedy of the commons: the generic problem of exploiting a shared limited resource [i]. Whereas a shared, limited resource can be used most efficiently by slow, prudent exploitation, selfish individuals can gain a disproportionate share of the total by rapid exploitation, and thus enjoy direct benefits at the collective expense of the group [d,g].

Virulence: presents a Pandora's box of interpretations, reflecting its often specialized and sometimes imprecise use in many different fields in biology [j]. In this article, we follow the consensus in evolutionary biology, where virulence tends to refer to the detrimental consequences of infection (namely loss of fitness) experienced by the host. By contrast, we use 'parasite trait' to refer to the exploitative behaviour of individual parasites. Note that even the summed parasite traits of an infrapopulation cannot entirely explain the pathogenic consequences for a host, as parasite-induced pathology is a complex product of host, parasite and environmental influences [k].

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versus 'collective' exploitation traits in parasites (Fig. 2). When the rate of exploitation is individually limited – that is, following standard 'tragedy' conceptions of virulence – an EVOLUTIONARILY STABLE parasite trait increases with decreasing relatedness among coinfecting parasites, up to a maximum level when within-host relatedness is minimal (Fig. 2c). If, however, the exploitation trait is a collective trait, we must consider instead a collective action scenario (Fig. 2a,b), whereby the parasite exploitation trait is maximal for perfectly related groups, expressing a group optimal level. As the genetic integrity of the group is lowered, so too is the profitability of group-level cooperation, resulting in an increasing temptation to cheat by opting-out of any communal action (but see [13,14] for an alternative explanation of reduced virulence under low relatedness owing to competitively driven mutual inhibition).

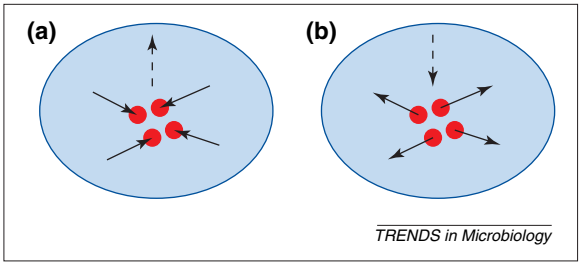

Fig. 1. A schematic diagram of 'tragedy of the commons' (a) and 'collective action' (b) parasite traits. Red dots represent parasites, the large blue circles represent hosts. Arrows represent the flow of energy or value with respect to the parasites. Solid arrows represent individual traits, dotted arrows represent group-level traits. (a) Tragedy of the commons: individual benefit, group costs. The individual benefit is derived from direct exploitation of a readily available common resource, the devaluation of which presents a collective cost of exploitation. (b) Collective action: individual cost, group benefits. Here, the individual benefit is derived by exploitation of a socially constructed or emergent common resource, the construction of which requires individual contributions to the common good.

To summarize the relatedness dimension of Fig. 2, one of two aspects of cooperation can be seen in highly related groups: collective action (Fig. 2a,b) or mutual restraint (Fig. 2c). Conversely, under conditions of low cooperation, we see solutions based on individual interest, characterized by either free-loading (Fig. 2a,b) or rapaciousness (Fig. 2c).

To interpret the impact of multiple infection on the evolution of virulence in a particular system, it is thus essential to understand whether the reproductive or exploitative rate of an individual parasite depends directly on the behaviour of the individual, or whether it is limited by the collective action of the coinfecting group. Taking the example of viral exploitation of a bacterial host, a mechanistically naive interpretation might view reproduction as an individually limited trait, and hence susceptible to the tragedy of the commons. However, given the mechanistic dependence of reproductive rate on the collective production of RNA replicase, we see that individual reproduction is group limited, while a new emphasis is placed on the underlying individual trait of replicase production.

Predicting parasite behaviour

Following the expectation of a qualitative biological distinction between the positive (collective action) and negative (tragedy of the commons) space of the individual parasite trait a^* (Box 1), Fig. 2 presents separate predictions for a 'collective' trait

Box 1. Collective action and the tragedy of the commons in a simple model

In a collective action model of virulence, contributing to the collective exploitation trait has a negative fitness impact on the individual, and a positive fitness impact on the group (and hence, indirectly, on every individual within the group). The approach taken here is to construct a fitness function, w, consisting of an individual and a group component in multiplicative form [a–d]:

$$w(a,\bar{a}) = (1-ca)(p+n\bar{a}) \qquad \text{[Eqn I]}$$

Here, a is the individual contribution to the collective action, and $w(a,\bar{a})$ is the fitness of an individual that invests a in collective action, in a group of size (or density) n, in which the average level of investment is $\bar{a}$. c represents the cost of cooperation and p represents passive fitness (the fitness of a non-contributing individual in a group of non-contributors). Thus, increasing a reduces the individual component of fitness ($1-ca$), but increases the group component ($p+n\bar{a}$). So long as a is limited to positive values, we have a model of collective action. However, by considering the negative space of a, we recover a classic tragedy of the commons model of virulence, where increasing the exploitation trait (increasing the negative magnitude of a) has a positive impact on the within-host fitness, and a negative impact on the among-host fitness [a,b]. Assuming that group members are related by a coefficient r, we can derive the following evolutionarily stable strategy of a (denoted a^*) from Eqn I (see [c,d] for details)

$$a^* = \frac{1+r(n-1)-cp}{c(1+r(n-1)+n)} \qquad \text{[Eqn II]}$$

The positive (collective action) and negative (tragedy of the commons) regions of a^* are plotted separately in Fig. 2, as a function of group size (n) and relatedness (r).

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(a^* constrained to be positive) and for a 'tragedy' trait (a^* constrained to be negative), as a function of parasite density (n) and relatedness (r). When passive fitness p (the fitness of a non-contributing individual in an group of non-contributors) is restricted to zero, a^* is constrained to be positive, and hence individual parasites are obliged to engage in collective action to gain any fitness, as otherwise the group resource is restricted to zero. The production of shared intracellular products in viral parasites provides an empirical example of a $p = 0$ scenario, as in the absence of, for example, RNA replicase production, viral infections cannot persist. For instance, 'defective interfering particles' [6–7,15] are short viral fragments that have a reproductive advantage when coinfecting with complete (cooperative) viral strains, but are unable to exploit the host in the absence of other infecting strains.

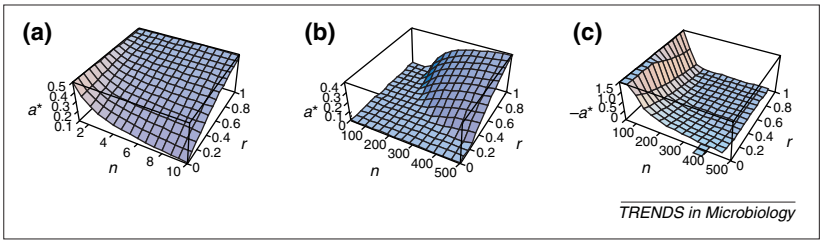

Fig. 2. Evolutionarily stable individual contributions to 'collective action' virulence [a^*; (a) and (b)] and 'tragedy of the commons' virulence [$-a^*$; (c)] as a function of relatedness (r) and intensity of infection (n). (a) Obligate collective action, $p = 0$ (e.g. RNA replicase); (b) threshold collective action, $p = 100$ (e.g. quorum-sensing traits); (c) tragedy of the commons, $p = 100$. In (a–c), $c = 1$.

Other empirical systems illustrate however that p can be greater than zero. For example, QUORUM SENSING bacteria regulate collective phenotypes in a facultative manner, with collective traits typically expressed only at high densities [16,17]. If p is positive (Fig. 2b), individuals have a 'ready-made' resource (p) to exploit directly and independently of others, making zero contribution the best strategy at low density. However, for a sufficiently high intensity of infection (n) and relatedness (r), the 'ready-made' resource p becomes insignificant relative to the potential for collective action. Turning to 'tragedy traits' (Fig. 2c), we see that the availability of a 'ready-made' resource (positive p) allows the operation of the tragedy of the commons (negative a^*).

Figure 2 also emphasizes the oft-neglected dimension of parasite intensity (n). Despite the longstanding realization that macroparasite pathology is highly dependent on the intensity of infection [17], and the increasing awareness of the role of microparasite density in determining virulence [18], evolutionary models have tended to ignore the numerical dimension of infection [19,20]. Looking at the 'tragedy' model of virulence (Fig. 2c), we see that individual exploitation ($-a^*$) is initially high, then declines with intensity (n). This is because the risk of a 'tragedy' is much lower for low intensities – one bacterium is unlikely to kill a host directly (although its more numerous descendents might). As intensity increases, the risk of a tragedy (i.e. over-exploitation of the host) increases, and hence the stable level of direct individual exploitation ($-a^*$) declines (it is important to remember that a^* represents only the behaviour of a single parasite). Similarly, in Fig. 2a, we see that given obligate collective action ($p = 0$), the individual contribution to the collective action declines with n. Here, individual trait expression declines owing to a 'many hands make light work' effect; that is, solitary parasites must contribute more to change the host environment than do individual parasites as part of a larger group. In Fig. 2b we see the emergence of a threshold to collective action following the introduction of non-zero p. At low densities, contributions are inefficient. However, following the accumulation of sufficient parasite biomass, contributing to the collective action trait becomes a worthwhile investment. In other words, parasites begin to take strategic control or 'responsibility' for the shared host phenotype as their proportional mass increases. In agreement with this threshold prediction, numerous bacterial species are known to regulate gene expression in a density-dependent manner, via quorum-sensing mechanisms [16,17]. The inter-bacterial signalling behaviour allowing the coordination of density-dependent phenotypic switching presents a further intriguing mix of cooperation and conflict. Brown and Johnstone [21] extend the model in Box 1 to predict that signal strength will peak for intermediate levels of relatedness.

Complications

How can we relate this simple framework to real biological examples, where virulence can be influenced by multiple parasite traits, some mediated individually, others collectively, and some even being the coordinated work of separate species working in tandem?

'...if the rate of exploitation is limited by a collective action trait, a collective action dynamic will provide a limiting barrier to any 'tragedy'-driven increase in exploitation rate at low relatedness.'

We argue above that if the rate of exploitation is limited by a collective action trait, a collective action dynamic will provide a limiting barrier to any 'tragedy'-driven increase in exploitation rate at low relatedness. For example, consider the bacterial plant pathogen *Erwinia carotovora*, and its production of exogenous digestive enzyme [22]. How would this exploitation trait be expected to change in response to increasing multiplicity of infection (and consequent decline in within-host relatedness)? The standard interpretation of virulence is that increased competition favours increased use of resources, and thus would predict increased enzymatic production to facilitate rapid host exploitation. By contrast, a collective action model would recognize that the production of a bacterial exo-enzyme is a 'common good', and hence raising its production offers no within-host gain whatsoever. Thus, we predict that increasing the multiplicity of infection of *E. carotovora* would tend to select for a decline in virulence, assuming digestive enzyme production is the major source of host pathology (Fig. 2b).

Caution is necessary however when considering the spatial scale of operation of a parasite trait. Following the logic developed in relation to Turner and Chao's [5] phage-virus study, all viral infections are at base governed by collective action dynamics, as viral particles cannot ensure exclusive access to their own gene products [7,11,23]. However, in viral infections of multicellular hosts, competition among independently exploiting strains or quasi-species [24] existing in different host tissues or cells might lead to a tragedy of the commons on a higher level of spatial organization. Similarly, the spatial dimension will be of importance in bacterial infections of multicellular hosts, as again a single host can experience numerous distinct pools of collective action, each providing an independent point of exploitation, which contribute on a larger spatial scale (namely that of the host) to a tragedy of the commons. Intriguingly, microparasitic infections of multicellular hosts can exhibit nested pools of collective action. For example, baculovirus infections in insects appear to engage in multicellular collective action through the production of moult-inhibiting products [25,26]. Thus, when considering the potential evolutionary consequence of a given regime of genetic mixing, it is important to consider the spatial structuring of the parasite population and its associated collective traits, particularly in multicellular hosts.

'...for many parasites, the maximal reproductive or exploitative rate of an individual is instead limited by the collective behaviour of coinfecting parasites, and thus is best characterized by a 'collective action' perspective.'

Finally, consider that the expression of certain 'collective' traits can reduce virulence, should the interests of host and parasite be sufficiently aligned [27]. For example, certain lysogenic phage carry resistance genes against antibiotics [28] and vertebrate host serum [29], thus reducing the negative impact of the lysogenic phage on its bacterial host. On a higher spatial scale, certain plasmids endow their bacterial hosts with anti-bacterial weaponry (colicins), allowing a specific bacterial strain to kill off its local competitors [30]. Thus, collective colicin production within a bacterial strain could reduce the bacterial density within the host. A similar dynamic of parasite-induced limitation of superinfection has been proposed in a macroparasitic context, and analyzed under the collective action framework [31].

Conclusions

Classic 'tragedy of the commons' models of virulence are based on the assumption that the individual parasite reproductive rate is defined by the behaviour of the individual, and potentially moderated by trade-offs with host-level traits, for example, mortality. However, a biological review of the mechanisms of host–parasite interactions suggests that for many parasites, the maximal reproductive or exploitative rate of an individual is instead limited by the collective behaviour of coinfecting parasites, and thus is best characterized by a 'collective action' perspective. Here, we present a simple predictive framework of parasite trait evolution under defined ecological conditions (in particular, within-host relatedness and density), which clearly distinguishes between individually and collectively limited mechanisms of exploitation.

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The alveolar macrophage: the Trojan horse of *Bacillus anthracis*

Chantal Guidi-Rontani

***Bacillus anthracis*, the causative agent of anthrax, has a particular strategy for invading the host and crossing the alveolar barrier. *B. anthracis* survives within alveolar macrophages, after germination within the phagolysosome, then enters the external medium where it proliferates. Recent data have shown that edema toxin and lethal toxin are the major genetic determinants mediating the survival of germinated spores within macrophages. Here, recent advances in the analysis of *B. anthracis* pathogenesis are summarized and future challenges discussed.**

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Bacillus anthracis is an endospore-forming [1], aerobic, rod-shaped bacterium. It colonizes its host using a repertoire of virulence determinants that cause bacteremia [2] and toxemia [3,4], and the result is systemic anthrax [5]. The germination of spores and the emergence of vegetative bacilli, in an environment allowing rapid out-growth into the host body, are essential in the early stages of pathogenesis to establish infection. Although anthrax is a well-known disease and was one of the first to be described and linked to its causative organism [1], our understanding of the cellular and molecular interactions between *B. anthracis* and the cells of the host immune system is far from complete, and many important questions remain. For many years, anthrax has been out of the spotlight but has recently been the focus of much attention owing to the emergence of the significant threat of *B. anthracis* being used as a potential agent of bioterrorism [6].

Macrophages as partners of *B. anthracis*

In its vegetative form, *B. anthracis* can survive as an extracellular pathogen and, owing to its anti-phagocytic poly-γ-D-glutamic acid capsule [7–10] and adenylate cyclase activity [11], can avoid ingestion by host phagocytes. However, *in vivo* experiments have demonstrated that, once inhaled, anthrax spores can reach the bronchioles and alveoli of the lung, and most spores are then rapidly and efficiently phagocytosed by alveolar macrophages via recruitment of F-actin [12–14].

In 1999, an article, highlighted by *Trends in Microbiology*, partially revealed the process that follows the phagocytosis of spores [14,15]. It described a novel strategy that *B. anthracis* uses to subvert a host immune cell to its advantage: the efficient germination of *B. anthracis* spores within the phagosomal compartment of bronchoalveolar macrophages following infection by inhalation (Fig. 1) [14]. Moreover, a germination operon (*gerX*) was associated with the germination of *B. anthracis* within macrophages and was shown to encode

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