THE ROYAL SOCIETY

Received 12 February 2004
Accepted 22 March 2004
Published online 4 June 2004

Bacteriocins, spite and virulence

Andy Gardner[1*], Stuart A. West[1] and Angus Buckling[2]

[1]*Institute of Cell, Animal and Population Biology, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK*
[2]*Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK*

There has been much interest in using social evolution theory to predict the damage to a host from parasite infection, termed parasite virulence. Most of this work has focused on how high kinship between the parasites infecting a host can select for more prudent exploitation of the host, leading to a negative relationship between virulence and parasite kinship. However, it has also been shown that if parasites can cooperate to overcome the host, then high parasite kinship within hosts can select for greater cooperation and higher growth rates, hence leading to a positive relationship between virulence and parasite kinship. We examine the impact of a spiteful behaviour, chemical (bacteriocin) warfare between microbes, on the evolution of virulence, and find a new relationship: virulence is maximized when the frequency of kin among parasites' social partners is low or high, and is minimized at intermediate values. This emphasizes how biological details can fundamentally alter the qualitative nature of theoretical predictions made by models of parasite virulence.

Keywords: social evolution; neighbour-modulated fitness; negative relatedness; Hamiltonian spite; scale of competition; interference competition

1. INTRODUCTION

There is a large theoretical literature applying evolutionary theory to explain the damage that parasites cause to their hosts (van Baalen & Sabelis 1995; Frank 1996; Gandon *et al.* 2001; Day & Burns 2003). Parasite virulence presents a fundamental trade-off in that parasites must deplete host resources to grow and transmit to new hosts, yet over-exploitation can result in host mortality and an associated reduction in resource availability (Frank 1996). This is the 'tragedy of the commons' (Hardin 1968), in which individuals are expected to display altruistic self-restraint only if they are sufficiently related to their group (Frank 1998). A classic result of virulence theory is that intensity of exploitation and hence damage to hosts correlates negatively with kinship among the parasites infecting a host (Hamilton 1972; Bremerman & Pickering 1983; Frank 1992, 1996). This occurs because a lower relatedness leads to greater competition for resources, which selects for faster growth rates to obtain a greater proportion of the host resources, and these higher parasite growth rates lead to higher virulence.

However, empirical support for this prediction is severely lacking (Herre 1993, 1995; Chao *et al.* 2000; Read & Taylor 2001; Davies *et al.* 2002; Griffin & West 2002; Read *et al.* 2002). One possible explanation for this is that variation in the underlying biological details can lead to alternative relationships (Frank 1996; Ganusov & Antia 2003; Schjørring & Koella 2003). In particular, it has been shown that if parasites can cooperate to overcome their host's defences then the opposite prediction is favoured—a positive relationship between parasite kinship and virulence (Chao *et al.* 2000; Brown *et al.* 2002; West & Buckling 2003). For example, West & Buckling (2003) modelled the evolution of the production of costly public goods (siderophores) that promote bacterial growth during iron starvation in an infection. Not surprisingly, the altruistic production of siderophores is expected to be maximized when kinship is highest, yet this leads to enhanced growth and therefore host damage precisely where previous theory predicted self-restraint and hence low virulence.

Just as altruistic behaviour can promote parasite growth and hence enhance virulence, it is reasonable to assume that spiteful interactions (interference competition) between parasites could reduce the vigour of an infection and associated host damage. We consider such a spiteful trait: bacteriocin production. Bacteriocins are the most abundant of a range of antimicrobial compounds facultatively produced by bacteria, and are found in all major bacterial lineages (Riley & Wertz 2002). They are a diverse family of proteins with a range of antimicrobial killing activity, many of which can be produced by a single bacterium, including enzyme inhibition, nuclease activity and pore formation in cell membranes (Reeves 1972; Riley & Wertz 2002). Unlike other antimicrobials, the lethal activity of bacteriocins is often (but not always) limited to members of the same species as the producer, suggesting a major role in competition with conspecifics (Riley *et al.* 2003). Intraspecific competition may also help to explain the observed variation in the types of bacteriocin produced by different strains of the same species. For example, at least 25 bacteriocins (colicins) have been identified in populations of *Escherichia coli*, with different populations producing unique combinations (Riley & Gordon 1999). Clone mates are protected from the toxic effects of bacteriocins by genetic linkage between the bacteriocin gene and an immunity gene that encodes a factor that deactivates the bacteriocin (Riley & Wertz 2002).

In addition to the benefits of bacteriocin production (killing competitors), there are also costs (Reeves 1972;

* Author for correspondence (andy.gardner@ed.ac.uk).

Chao & Levin 1981; Kerr *et al.* 2002). This cost may simply be a diversion of resources from other cellular functions, but in many Gram-negative bacteria, such as *E. coli*, cell death is required for the release of bacteriocins (Reeves 1972; Riley & Wertz 2002). Such costs (and costs associated with bacteriocin immunity) are critical for coexistence, between bacteriocin-producing, sensitive and resistant strains (Czárán *et al.* 2002; Kerr *et al.* 2002; Czárán & Hoekstra 2003). We investigate how key parameters affect the relative costs and benefits of bacteriocin production, hence the level favoured by natural selection, and the impact this has on disease virulence. Specifically, we consider how bacteriocin production evolves in response to the average kinship (r) of competing bacteria and the scale of competition relative to the effective range of bacteriocins (a).

2. MODELS, METHODS AND ANALYSES

(a) *Simplest scenario*

We first consider a social arena, defined as the spatial range of bacteriocin warfare, comprising n equally abundant lineages drawn independently from the asexually reproducing bacterial population. A proportion $r = 1/n$ of the bacteria within a focal bacterium's social arena are its clone-mates, or 'kin'. The remaining $1 - r$ are derived from the other $n - 1$ lineages, and are 'non-kin'. Using a game theoretic approach, we consider the fitness of a vanishingly rare mutant that allocates an amount of resources y into bacteriocin production within a population with average allocation z, so as to determine the 'unbeatable' (Hamilton 1967) or 'evolutionarily stable' (Maynard Smith & Price 1973) allocation strategy y^*. An amount of bacteriocin ry within the social arena is attributable to the focal lineage, and rz to each of the other lineages. The focal lineage is then subjected to an amount $(1 - r)z$ of unrelated bacteriocin to which it is susceptible, and for each of the $n - 1$ other lineages, $(1 - r)z + r(y - z)$. A lineage picked at random from the population as a whole experiences, on average, $(1 - r)z$ unrelated bacteriocin. Lineages are immune to their own bacteriocins, and although resistance (non-susceptibility of a lineage to a bacteriocin which it does not itself produce) is not explicitly discussed in this model, the resulting reduction in susceptibility can be regarded as included in the general growth functions. The growth rate of a lineage, G, is given by the sum of two components, H and I. H reflects the cost of bacteriocin production, being a positive, decreasing function of the focal lineage's allocation to bacteriocin production, y. Our predictions rely on no specific form for H; when a specific relationship is required for illustrative purposes (figures 1–3), we use $H = 1 - y$. I models the reduction in growth owing to mortality by unrelated bacteriocins, being a positive, decreasing, linear or decelerating function of the amount (Y) of unrelated bacteriocin it is subjected to. Our predictions rely on no specific form for I; when a specific relationship is required for illustrative purposes (figures 1–3), we use $I = 1 - Y^{1/2}$. We combine the terms H and I additively to give overall growth ($G = H + I$) for mathematical convenience, as it allows greater tractability than using a multiplicative scheme ($G = H \times I$), and does not qualitatively change the results (see Appendix B). Using the construction of Frank (1998), fitness is determined by the growth of the lineage relative to the average competitor of that lineage:

$$w = \frac{G_{\text{focal}}}{a\,G_{\text{local}} + (1 - a)\,G_{\text{global}}}. \qquad (2.1)$$

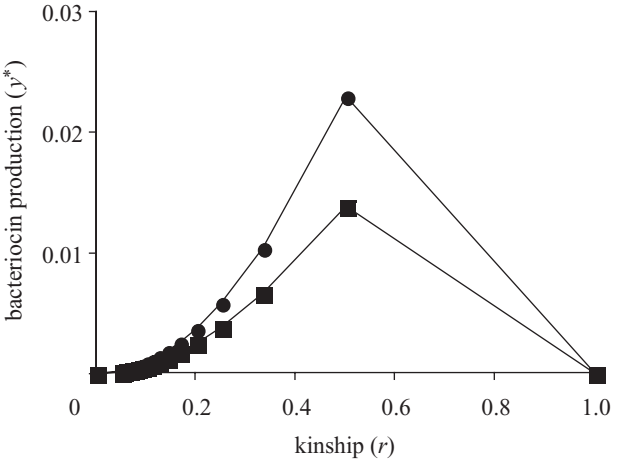

Figure 1. The ESS production of bacteriocins (y^*) as a function of the average kinship (r) between bacteria. Values are obtained numerically using the model described in § 2a, assuming that bacterial growth is the sum of growth components $H = 1 - y$ and $I = 1 - Y^{1/2}$ (where the focal bacterium produces an amount y of its own bacteriocins, and receives an amount Y from its social partners) and the intensity of local competition which is local is $a = 0.5$ (filled squares) and 0.6 (filled circles). Intermediate kinship (r) and increasingly local competition (high a) favour enhanced bacteriocin production.

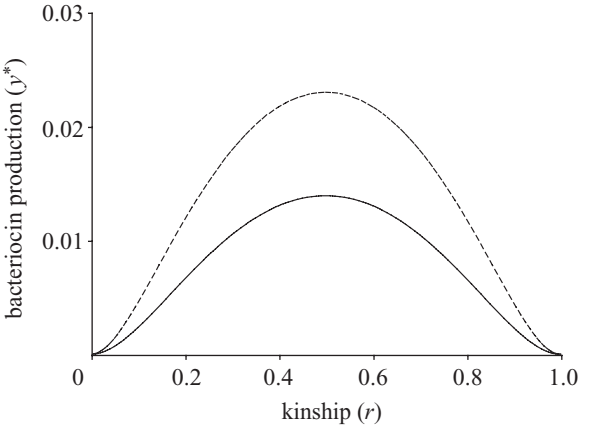

Figure 2. The ESS production of bacteriocin (y^*) as a function of the average kinship (r) between bacteria. Values are obtained numerically using the two-lineage model described in Appendix A, assuming that bacterial growth is the sum of growth components $H = 1 - y$ and $I = 1 - Y^{1/2}$ (where the focal bacterium produces an amount y of its own bacteriocins, and receives an amount Y from its social partners) and the intensity of local competition which is local is $a = 0.5$ (solid line) or $a = 0.6$ (dotted line). Intermediate kinship (r) and increasingly local competition (high a) favour enhanced bacteriocin production.

The parameter a defines the (spatial) scale at which competition for resources takes place. This model therefore allows competition for resources and bacteriocin interaction to take place at different scales. Specifically, a proportion a of competition for resources occurs locally, within the scale of bacteriocin interaction, and the $(1 - a)$ remainder occurs globally. At the extremes: if $a = 1$ then competition for resources and bacteriocin interaction occur at the same scale (soft selection at the level of the social group); if $a = 0$ then competition is at the global level

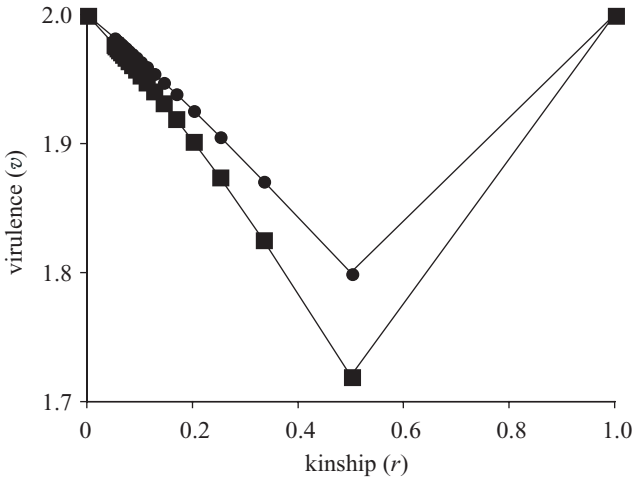

Figure 3. The virulence (v) as a function of the average kinship (r) between bacteria. Values are obtained numerically using the host mortality model described in § 2b, assuming that bacterial growth is the sum of growth components $H = 1 - y$ and $I = 1 - Y^{1/2}$ (where the focal bacterium produces an amount y of its own bacteriocins, and receives an amount Y from its social partners), host survival is $S = 3 - G_{\text{host}}$ (where G_{host} is the overall bacterial growth in the host), the intensity of local competition is $a = 0.5$, and the range of bacteriocin warfare with respect to the whole infection is $b = 0.1$ (filled circles) and 0.2 (filled squares). Virulence is minimized at intermediate kinship (r) and when the range of bacteriocin warfare (b) is large.

(hard selection at the level of the social group). G_{focal}, G_{local} and G_{global} are, respectively, the growth rate of the focal lineage, the local average and the global average. These are, in full

$$\begin{aligned} G_{\text{focal}} &= H[y] + I[(1-r)z], \\ G_{\text{local}} &= r\,(H[y] + I[(1-r)z]) + (1-r)(H[z] + I[(1-r)z \\ &\qquad + r(y-z)]), \\ G_{\text{global}} &= H[z] + I[(1-r)z]. \end{aligned} \tag{2.2}$$

Equations (2.1) and (2.2) illustrate the fundamental trade-off in our model. Bacteriocin production by the focal lineage is: (i) costly, because it lowers the growth rate of the focal lineage (G_{focal}); and (ii) beneficial, because it lowers the growth rate of competitors G_{local}.

Employing the direct fitness maximization technique of Taylor & Frank (1996; Frank 1998), we obtain the following results (details in Appendix A; numerical examples are given in figure 1).

Result 1: enhanced bacteriocin production is favoured at intermediate kinship (r). The evolutionarily stable strategy (ESS) is $y^* = 0$ at $r = 0$ and 1, and is maximized somewhere in the range $0 < r < 1$. When the focal lineage occupies only a tiny proportion ($r \to 0$) of the social arena, its impact on competitor growth is negligible, and hence the benefit through competitor killing does not outweigh the cost of bacteriocin production. When the focal lineage dominates the social group ($r \to 1$), the density of cells susceptible to its bacteriocin is too low for the benefit of competitor killing to outweigh the production costs.

Result 2: enhanced bacteriocin production is favoured as the scale of competition a is increased (and hence competition for resources becomes more local) for all $0 < r < 1$. This occurs because fitness can be enhanced in two ways: (i) maximizing own growth (G_{focal}); and (ii) reducing the growth of local competitors (G_{local}). When competition is entirely global ($a = 0$), there is no benefit in reducing the growth of local competitors, so that the ESS is the strategy that maximizes focal growth (by reducing bacteriocin production). As competition becomes more local ($a > 0$), production of bacteriocin is increasingly favoured so as to reduce the growth of the local competitors.

We also consider a model in which the abundance of the focal lineage can vary continuously over the range $0 \leqslant r \leqslant 1$, and the other cells all belong to one other lineage (see Appendix A and figure 2). We recover the same results, finding that ESS bacteriocin production is maximized at intermediate kinship (at $r = 1/2$, because of the symmetry of this model) and increases as competition becomes more localized (i.e. as a increases).

As is often the case (Taylor & Frank 1996; Frank 1998), inspection of the direct marginal fitness (equation (A 1)) yields a form of Hamilton's (1963) rule $RB > C$ (equation (A 2)). In this: (i) relatedness is negative and given by $R = -(ar)/(1 - ar)$; (ii) the negative 'benefit', summed over all recipients, is $B = (1 - r)I'[(1 - r)z]$ where $I'[Y]$ is the derivative $\mathrm{d}I[Y]/\mathrm{d}Y$ and represents the marginal reduction in growth of a lineage which is poisoned by an amount Y of foreign bacteriocins. To understand how a negative relatedness can arise, we will use the result of Queller (1994) that average relatedness to one's competitors is zero. Recalling that the scale of competition (a) is defined as the proportion of competition which is local, consider an arena of competition in which a proportion of competitors a are social partners, and of these a proportion r belong to the focal lineage. Then a proportion ar of competitors are clonally related to the spiteful actor by 1, and a proportion $1 - ar$ are related by some unknown coefficient R. Applying Queller's insight, we know that $ar \times 1 + (1 - ar) \times R = 0$, and rearranging we obtain $R = -(ar)/(1 - ar)$. Hence:

Result 3: the evolution of bacteriocin production involves a negative relatedness between actor and recipient, and hence fits Hamilton's (1970) original definition of a spiteful behaviour. Relatedness between non-kin social partners is given by $R = -(ar)/(1 - ar)$, where a is the proportion of competition that is local, and r is the proportion of social partners that are clonal kin. This equation gives negative values for relatedness, except when either (or both) a and r are zero, in which case relatedness equals zero.

(b) *Host mortality*

The above model is appropriate for free-living bacteria, bacteria grown on agar plates, or parasitic bacteria in which host mortality does not influence the ESS production of bacteriocin. For parasitic bacteria, this would be appropriate when the extra host mortality due to the infection impinges very little upon bacterial success, or when there are many social groups within the host, such that any lineage's growth rate has a negligible impact on the mortality of the host. A simple model, relaxing these assumptions, considers that direct fitness of the focal lineage is given by the product $S \times T$, where S represents host survival (i.e. the time over which transmission is possible) and is a linearly decreasing function of the average growth rate of lineages in the host. T is the transmission rate achieved by the focal lineage, i.e. its growth rate relative to competitors, the fitness measure given by equation (2.1). A parameter, b, is introduced to denote the proportion of the bacterial population within the host that is in the focal arena of social (bacteriocin) interaction; $b = 0$ corresponds to when the social arena comprises a vanishingly small

proportion of the total infection, and $b = 1$ corresponds to the arena of bacteriocin interaction being the entire infection. As in our first model, we assume n equally abundant lineages. The appropriate fitness function is

$$w = S[G_{\text{host}}]\frac{G_{\text{focal}}}{aG_{\text{local}} + (1 - a)\,G_{\text{global}}}, \tag{2.3}$$

where the growth rate of a random lineage within the host is on average

$$G_{\text{host}} = bG_{\text{local}} + (1 - b)\,G_{\text{global}}. \tag{2.4}$$

Virulence (v) can be defined as the reduction in S relative to a host with zero bacterial growth ($G_{\text{host}} = 0$), i.e. $v = S[0] - S[G_{\text{host}}]$. The following result is obtained (see Appendix A for details, and figure 3 for numerical examples).

Result 4: virulence (v) is maximized at the extremes of relatedness ($r = 0$ and $r = 1$), and is minimized at intermediate values $0 < r < 1$. This is because of the maximization of bacteriocin production at intermediate values of r, such that absolute growth of bacteria is reduced here but not at more extreme values, so that virulence is more pronounced whenever bacteria tend to socialize mostly, or not at all, with their kin.

3. DISCUSSION

We have shown that the production of bacteriocin is expected to be enhanced when kinship (r) is of intermediate value (result 1; figures 1 and 2). Because bacteriocin production is expected to correlate with low bacterial growth rates, virulence will tend to be minimized at intermediate r and maximized when bacteria compete only with non-kin ($r = 0$) or only with kin ($r = 1$). We therefore predict a U-shaped relationship between virulence and kinship (result 4; figure 3), contrary to previous models that variously predict monotonically increasing or decreasing virulence as kinship is increased. This emphasizes that the qualitative outcome of virulence evolution crucially depends on the biological details, such as whether parasites are able to improve their success through prudent growth (Frank 1996), or cooperative contributions to public goods (Brown *et al.* 2002; West & Buckling 2003), or through anti-competitor toxin production.

Our result is intuitive if we consider that when kinship (r) is low the influence of the focal lineage on the growth of its social partners will be negligible, and so reduced allocation of resources into bacteriocin production is favoured. By contrast, when kinship is high, the proportion of cells in the social arena that are susceptible to bacteriocin killing is small, and thus the benefit of producing bacteriocin is less than the cost that this entails. At intermediate kinship, bacteriocin production is favoured because competition with non-relatives is important, and bacteriocin production by the focal lineage can significantly decrease the growth of the non-competitors. Result 2, that the ESS bacteriocin production is an increasing function of the degree to which competition is local (a; figures 1 and 2), is also intuitive in that when competition is increasingly local the benefits accrued by reducing the growth of local competitors are enhanced.

The costly allocation of resources into bacteriocin production qualifies as an example of Hamiltonian spite (Hamilton 1970, 1996; Hurst 1991; Foster *et al.* 2001; Gardner & West 2004). It is well accepted that altruism can be adaptive despite a direct fitness cost provided the beneficiary of altruism is sufficiently positively related to the actor (i.e. a positive R and a positive B, and $RB > C$). Hamiltonian spite is when a costly behaviour is favoured because it has a cost to the recipient (negative B), and the recipient is negatively related to the actor (negative R, and $RB > C$). How can negative relatedness arise? Negative relatedness to some individuals is inevitable when positively related individuals exist in the same competitive arena. The reason for this is that because the relatedness of an actor to a randomly chosen individual from its competitive arena is, on average, zero (Queller 1994), the existence of positive relations within that arena implies the existence of negatively related competitors (Result 3). In this situation, spiteful behaviour will be favoured if it can be preferentially directed at these negatively related competitors, and $RB > C$ is satisfied. The specificity of bacteriocin action allows it to potentially fill this criterion, because it will preferentially harm non-relatives who are not resistant to that particular bacteriocin; i.e. bacteriocins harm individuals who are negatively related to the producer. Although the anti-competitor function of the bacteriocins suggests that this is selfishness at the level of the clonal lineage, it is certainly spiteful at the level of the self-destructing bacterium producing the toxins.

To conclude, we have shown theoretically how kinship and the scale of competition determine levels of bacteriocin production favoured by natural selection. Contrary to previous work, we find a U-shaped relationship between kinship and virulence. The results are qualitatively the same whether bacteria have fixed strategies for bacteriocin production or if bacteriocin production is facultatively adjusted in response to kin recognition. These predictions could be tested by: (i) correlating bacteriocin production with average kinship in natural populations; or (ii) experimentally evolving bacteria under different degrees of kinship and scales of competition. Furthermore, our predictions are not limited to bacteriocin production by bacteria. A variety of microbes, including yeasts (see Schmitt & Breinig 2002) and halophilic archea (see Cheung *et al.* 1997) are known to produce toxins that tend to target conspecifics.

We thank N. Barton and three anonymous reviewers for comments. Funding was provided by BBSRC, NERC and The Royal Society.

APPENDIX A

(a) *Simplest scenario*

Substituting equation (2.2) into equation (2.1) we obtain fitness function $w[y,z]$. If we assume only minor variants ($y \approx z$; Taylor & Frank 1996) the marginal fitness is found to be

$$\left.\frac{\mathrm{d}w}{\mathrm{d}y}\right|_{y=z} = \frac{(1 - ar)H'[z] - ar\,(1 - r)I'[(1 - r)z]}{H[z] + I[(1 - r)z]}. \tag{A 1}$$

Where $H' < 0$ is the derivative of H with respect to its parameter (e.g. y in the instance of the mutant), and may be interpreted as the marginal cost ($-C$) of producing bacteriocins. $I' < 0$ is the derivative of I with respect to its parameter (e.g. $(1 - r)z$ for the amount of bacteriocin attacking the focal mutant), and is the negative 'benefit'

accrued by the recipient of spiteful behaviour—summing over all the recipients, the benefit is $B = (1-r)I'[(1-r)z]$. Increased bacteriocin production (y) is favoured whenever $dw/dy > 0$ is satisfied, yielding Hamilton's rule:

$$-\frac{ar}{1-ar}B > C. \tag{A 2}$$

Substituting $r = 0$ into equation (A 1) obtains $H'[z]/(H[z] + I[z])$, which is negative and hence $y^* = 0$. When $r = 1$, equation (A 1) becomes $(1-a)H'[z]/(H[z] + I[z])$ which is negative and so $y^* = 0$. When $a = 0$, equation (A 1) gives $H'[z]/(H[z]+I[(1-r)z])$, which is negative so that $y^* = 0$. Therefore, the presence of more than one lineage ($0 < r < 1$) and some degree of local competition ($a > 0$) are essential for non-zero allocation to bacteriocin production. If we denote the right-hand side (RHS) of equation (A 1) by $\mathcal{J}$, then the ESS $z = y^*$ satisfies $\mathcal{J} = 0$. Using implicit differentiation, we can write

$$\frac{dy^*}{dr} = -\frac{\delta\mathcal{J}/\delta r}{\delta\mathcal{J}/\delta y^*}, \tag{A 3}$$

where δ denotes partial derivatives. For y^* to be convergence stable (i.e. in a population close to y^*, mutants closer to y^* are favoured by selection), the denominator on the RHS of equation (A 3) must be negative (Taylor 1996). Hence, assuming convergence stability, dy^*/dr has the same sign as $\delta\mathcal{J}/\delta r$ (Pen 2000). Evaluating the partial derivative at $r = 0$ (and hence $y^* = 0$) yields $-a(H[0] + I[0])(H'[0] + I'[0])/(H[0] + I[0])^2$, which is positive when $a > 0$. This indicates that when there is some degree of local competition, and intermediate relatedness, bacteriocin production will be nonzero. Using the same procedure, we may find the partial derivative of $\mathcal{J}$ with respect to the scale of competition, a:

$$\frac{\delta\mathcal{J}}{\delta a} = -\frac{rH'[y^*] + r(1-r)I'[(1-r)y^*]}{H[y^*] + I[y^*]}, \tag{A 4}$$

which is positive for all $0 < r < 1$, and hence bacteriocin production is an increasing function of the scale of competition (a) when kinship is intermediate.

We now relax the assumption of equally abundant lineages, looking now at the situation where only two lineages occupy the social arena, so that the focal lineages comprise a proportion r or $1-r$ of the bacterial cells with equal probability. The appropriate fitness function is then

$$w = r\frac{G_{\text{focal1}}}{aG_{\text{local1}} + (1-a)\,G_{\text{global}}} + \frac{(1-r)\,G_{\text{focal2}}}{aG_{\text{local2}} + (1-a)\,G_{\text{global}}} \tag{A 5}$$

where

$$\begin{aligned}
G_{\text{focal1}} &= H[y] + I[(1-r)z],\\
G_{\text{focal2}} &= H[y] + I[rz],\\
G_{\text{local1}} &= r\,(H[y] + I[(1-r)z]) + (1-r)(H[z] + I[ry]),\\
G_{\text{local2}} &= (1-r)\,(H[y] + I[r\,z]) + r(H[z] + I[(1-r)y]),\\
G_{\text{global}} &= H[z] + r\,I[(1-r)z] + (1-r)\,I[rz].
\end{aligned} \tag{A 6}$$

Following the same procedure as before, we obtain

$$\left.\frac{dw}{dy}\right|_{y=z} = \left\{\begin{array}{l} ar(1-r)\begin{pmatrix} r(H[z] + I[(1-r)z])I'[rz] \\ +(1-r)(H[z] + I'[rz])I'[(1-r)z] \end{pmatrix} \\ +\begin{pmatrix} (1-a(1-2r(1-r)))H[z] \\ +r(1-ar)I[(1-r)z] \\ +(1-r)(1-a(1-r))I[rz] \end{pmatrix}H'[z] \end{array}\right\} \Big/ \{H[z] + rI[(1-r)z] + (1-r)I[rz]\}^2. \tag{A 7}$$

Setting $r \to 0$ yields $(1-a)H'[z]/(H[z] + I[0])$ which is always negative and hence $y^* = 0$ at $r = 0$. Setting $r \to 1$ yields $(1-a)H'[z]/(H[z] + I[0])$ which is always negative, so $y^* = 0$ at $r = 1$. And when $a \to 0$, we obtain $H'[z]/(H[z] + rI[(1-r)z] + (1-r)I[rz])$ which is always negative, so that $y^* = 0$ when $a = 0$.

As before, if we define $\mathcal{J}$ as the RHS of equation (A 7) when $z = y^*$, then it is easy to show that for $a > 0$, $\delta\mathcal{J}/\delta r = dy^*/dr = 0$ is satisfied for only $r = 1/2$. Since $y^* = 0$ at $r = 0$ and $r = 1$, and assuming no discontinuities over the range of r, we can conclude that y^* monotonically increases over the range $0 < r < 1/2$ and montonically decreases over the range $1/2 < r < 1$.

The partial derivative of $\mathcal{J}$ with respect to the scale of competition is $\delta\mathcal{J}/\delta a = -(r(1-r)(r(H[y^*] + I[(1-r)]) \times I'[ry^*] + (1-r)(H[y^*] + I[ry^*])I'[(1-r)y^*]) + (1 - 2r(1-r))H[y^*] + r^2I[(1-r)y^*] + (1-r)^2I[ry^*])H'[y^*])/(H[y^*] + rI[(1-r)y^*] + (1-r)I[ry^*])^2$, which is positive for all $0 < r < 1$, and hence bacteriocin production is an increasing function of the scale of competition (a) at intermediate kinship.

(b) *Host mortality*

Previously we constructed a fitness function (equation (2.3)) appropriate to the situation where bacterial growth impacts upon host mortality (virulence) and hence introduces a novel selection pressure. We also introduced a parameter b scaling the social arena with respect to the host. If $b = 0$, so that the social arena comprises a vanishing proportion of the bacterial population within the host, then $G_{\text{host}} = G_{\text{global}}$ and S is a constant with respect to y, so that marginal fitness is given by equation (A 1). For $b > 0$, and assuming only minor variants ($y \approx z$, $G_{\text{focal}} \approx G_{\text{local}} \approx G_{\text{global}} \approx G_{\text{host}} \approx G$), marginal fitness is

$$\frac{dw}{dy} = S'[G]rb(H'[z] + (1-r)I'[(1-r)z]) + S[G]\frac{(1-ar)H'[z] - ar(1-r)I'[(1-r)z]}{G}. \tag{A 8}$$

The second component on the RHS is proportional to the marginal fitness (equation (A 1)), and represents the trade-off between the cost and competitor-killing capabilities of bacteriocins. When $a = 0$, this component reduces to $(S[G]H'[z])/G$, which is always negative, reflecting the disadvantage of spite when competition is global. The first component, positive and proportional to rb, is the selection pressure for enhanced killing and costly production when growth of the focal lineage and its neighbours impact non-trivially upon host mortality. As r tends to zero, marginal fitness is negative ($S[G]H'[z]/G$) as the behaviour of the focal lineage has no impact on host mortality and there is no advantage to be had from directing spite at local competitors (relatedness to non-kin in the social arena is zero). At $r = 1$, the second component is

negative ($S[G](1-a)H'[z]/G$) reflecting the fitness cost of bacteriocin production, and the first component is positive ($S'[G]H'[z]$) reflecting the enhanced fitness due to the reduction in host mortality. Note that this positive pressure is due entirely to the costs of bacteriocin production, and not through its bacteriocidal activity; this is due to an artificiality in the model such that the bacteria have no means of reducing own growth other than producing costly bacteriocin. Because no gain in terms of competitor killing is to be had from producing bacteriocins at $r=1$, we expect $y^*=0$.

If $y^*=0$ at $r=0$ and 1, then since H and I are decreasing functions of y^*, it is here that $G_{\text{host}}=H+I$ is maximized. Because S decreases with increasing G_{host}, S is minimized at $r=0$, 1. If we define virulence as the reduction in host survival relative to that for a host in which bacterial growth is zero ($v=S_{\max}-S$), then virulence is maximized when S is minimized ($v_{\max}=S_{\max}-S_{\min}$), i.e. at the extremes of relatedness, $r=0$ and $r=1$.

When a and b are both zero, so that there is no selection for spite nor for reduced virulence, equation (A 8) reduces to $(S[G]H'[z])/G$ which is negative and hence $y^*=0$.

APPENDIX B

Relaxing the assumption of additive growth components, and making no further assumptions about the components of growth beyond bacteriocin production reducing the growth of the focal lineage (G_{focal}) and its non-kin social partners (G_{social}), we can recover the major predictions made in this study. Consider the fitness function (equation (2.1)). Marginal fitness can be written

$$\frac{\mathrm{d}w}{\mathrm{d}y}=\frac{(aG_{\text{local}}+(1-a)G_{\text{global}})\dfrac{\mathrm{d}G_{\text{focal}}}{\mathrm{d}y}-G_{\text{focal}}\dfrac{\mathrm{d}(aG_{\text{local}}+(1-a)G_{\text{global}})}{\mathrm{d}y}}{(aG_{\text{local}}+(1-a)G_{\text{global}})^2}. \tag{B 1}$$

Assuming only minor variants, so that $y\approx z$, and $G_{\text{focal}}\approx G_{\text{social}}\approx G_{\text{local}}\approx G_{\text{global}}\approx G$, we have

$$\frac{\mathrm{d}w}{\mathrm{d}y}=\left((1-ar)\frac{\mathrm{d}G_{\text{focal}}}{\mathrm{d}y}-a(1-r)\frac{\mathrm{d}G_{\text{social}}}{\mathrm{d}y}\right)/G. \tag{B 2}$$

Fitness increases with enhanced bacteriocin production when $\mathrm{d}w/\mathrm{d}y>0$. $\mathrm{d}G_{\text{focal}}/\mathrm{d}y$ is negative owing to the production costs of bacteriocin, and $\mathrm{d}G_{\text{social}}/\mathrm{d}y$ is negative because non-kin social partners experience higher mortality as bacteriocin production by the focal lineage is increased. Equation (B 2) therefore demonstrates the trade-off between the direct cost of bacteriocin production and the benefit of competitor killing. The benefit is zero when $a=0$ and/or when $r=1$, so that marginal fitness is $\{(1-ar)\mathrm{d}G_{\text{focal}}/\mathrm{d}y\}/G<0$ for all y, meaning that the ESS bacteriocin production is at $y^*=0$. Also, the impact of the focal lineage's bacteriocin on competitor growth approaches zero as the focal lineage accounts for a vanishing proportion of the social group, i.e. at $r=0$, $\mathrm{d}G_{\text{social}}/\mathrm{d}y=0$, and so here the marginal fitness is negative, and $y^*=0$. Therefore, regardless of the precise details describing how the growth of the focal lineage and its non-kin social partners decline with enhanced bacteriocin production, provided they do decline, we can state that the ESS is $y^*=0$ when kinship is zero or complete ($r=0$, 1) and when competition is entirely global ($a=0$).

REFERENCES

Bremerman, H. J. & Pickering, J. 1983 A game-theoretical model of parasite virulence. *J. Theor. Biol.* **100**, 411–426.

Brown, S. P., Hochberg, M. E. & Grenfell, B. T. 2002 Does multiple infection select for raised virulence? *Trends Microbiol.* **10**, 401–405.

Chao, L. & Levin, B. R. 1981 Structured habitats and the evolution of anticompetitor toxins in bacteria. *Proc. Natl Acad. Sci. USA* **78**, 6324–6328.

Chao, L., Hanley, K. A., Burch, C. L., Dahlberg, C. & Turner, P. E. 2000 Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. *Q. Rev. Biol.* **75**, 261–275.

Cheung, J., Danna, K., O'Connor, E., Price, L. & Shand, R. 1997 Isolation, sequence, and expression of the gene encoding halocin H4, a bacteriocin from the halophilic archaeon *Haloferaz mediterranei* R4. *J. Bacteriol.* **179**, 548–551.

Czárán, T. L. & Hoekstra, R. F. 2003 Killer-sensitive coexistence in metapopulations of micro-organisms. *Proc. R. Soc. Lond.* B **270**, 1373–1378. (DOI 10.1098/rspb.2003.2328.)

Czárán, T. L., Hoekstra, R. F. & Pagie, L. 2002 Chemical warfare between microbes promotes biodiversity. *Proc. Natl Acad. Sci. USA* **99**, 786–790.

Davies, C. M., Fairbrother, E. & Webster, J. P. 2002 Mixed strain schistosome infections of snails and the evolution of parasite virulence. *Parasitology* **124**, 31–38.

Day, T. & Burns, J. G. 2003 A consideration of patterns of virulence arising from host–parasite coevolution. *Evolution* **57**, 671–676.

Foster, K. R., Wenseleers, T. & Ratnieks, F. L. W. 2001 Spite: Hamilton's unproven theory. *Annls Zool. Fennici* **38**, 229–238.

Frank, S. A. 1992 A kin selection model for the evolution of virulence. *Proc. R. Soc. Lond.* B **250**, 195–197.

Frank, S. A. 1996 Models of parasite virulence. *Q. Rev. Biol.* **71**, 37–78.

Frank, S. A. 1998 *Foundations of social evolution*. Princeton University Press.

Gandon, S., Mackinnon, M. J., Nee, S. & Read, A. F. 2001 Imperfect vaccines and the evolution of pathogen virulence. *Nature* **414**, 751–756.

Ganusov, V. V. & Antia, R. 2003 Trade-offs and the evolution of virulence of microparasites: do details matter? *Theor. Popul. Biol.* **64**, 211–220.

Gardner, A. & West, S. A. 2004 Spite and the scale of competition. *J. Evol. Biol.* **17**. (In the press.)

Griffin, A. S. & West, S. A. 2002 Kin selection: fact and fiction. *Trends Ecol. Evol.* **17**, 15–21.

Hamilton, W. D. 1963 The evolution of altruistic behaviour. *Am. Nat.* **97**, 354–356.

Hamilton, W. D. 1967 Extraordinary sex ratios. *Science* **156**, 477–488.

Hamilton, W. D. 1970 Selfish and spiteful behaviour in an evolutionary model. *Nature* **228**, 1218–1220.

Hamilton, W. D. 1972 Altruism and related phenomena, mainly in social insects. *A. Rev. Ecol. Syst.* **3**, 193–232.

Hamilton, W. D. 1996 *Narrow roads of geneland I: evolution of social behaviour*. Oxford: Freeman.

Hardin, G. 1968 The tragedy of the commons. *Science* **162**, 1243–1248.

Herre, E. A. 1993 Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* **259**, 1442–1445.

Herre, E. A. 1995 Factors influencing the evolution of virulence: nematode parasites of fig wasps as a case study. *Parasitology* **111**, S179–S191.

Hurst, L. D. 1991 The evolution of cytoplasmic incompatibility or when spite can be successful. *J. Theor. Biol.* **148**, 269–277.

Kerr, B., Riley, M. A., Feldman, M. W. & Bohannan, B. J. M. 2002 Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors. *Nature* **418**, 171–174.

Maynard Smith, J. & Price, G. R. 1973 The logic of animal conflict. *Nature* **246**, 15–18.

Pen, I. 2000 Reproductive effort in viscous populations. *Evolution* **54**, 293–297.

Queller, D. C. 1994 Genetic relatedness in viscous populations. *Evol. Ecol.* **8**, 70–73.

Read, A. F. & Taylor, L. H. 2001 The ecology of genetically diverse infections. *Science* **292**, 1099–1102.

Read, A. F., Mackinnon, M. J., Anwar, M. A. & Taylor, L. H. 2002 Kin selection models as explanations of malaria. In *Virulence management: the adaptive dynamics of pathogen–host interactions* (ed. U. Dieckmann, J. A. J. Metz, M. W. Sabelis & K. Sigmund), pp. 165–178. Cambridge University Press.

Reeves, P. 1972 *The bacteriocins*. New York: Springer.

Riley, M. A. & Gordon, D. M. 1999 The ecological role of bacteriocins in bacterial cooperation. *Trends Microbiol.* **7**, 129–133.

Riley, M. A. & Wertz, J. E. 2002 Bacteriocins: evolution, ecology, and application. *A. Rev. Microbiol.* **56**, 117–137.

Riley, M. A., Goldstone, C. M., Wertz, J. E. & Gordon, D. 2003 A phylogenetic approach to assessing the targets of microbial warfare. *J. Evol. Biol.* **16**, 690–697.

Schjørring, S. & Koella, J. C. 2003 Sub-lethal effects of pathogens can lead to the evolution of lower virulence in multiple infections. *Proc. R. Soc. Lond.* B **270**, 189–193. (DOI 10/1098/rspb.2002.2233.)

Schmitt, M. J. & Breinig, F. 2002 The viral killer system in yeast: from molecular biology to application. *FEMS Microbiol. Rev.* **26**, 257–276.

Taylor, P. D. 1996 Inclusive fitness arguments in genetic models of behaviour. *J. Math. Biol.* **34**, 654–674.

Taylor, P. D. & Frank, S. A. 1996 How to make a kin selection model. *J. Theor. Biol.* **180**, 27–37.

van Baalen, M. & Sabelis, M. W. 1995 The scope for virulence management—a comment on Ewald's view on the evolution of virulence. *Trends Microbiol.* **3**, 414–416.

West, S. A. & Buckling, A. 2003 Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. Lond.* B **270**, 37–44. (DOI 10.1098/rspb.2002.2209.)

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.