KIN SELECTION AND PARASITE EVOLUTION: HIGHER AND LOWER VIRULENCE WITH HARD AND SOFT SELECTION

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ABSTRACT

Conventional models predict that low genetic relatedness among parasites that coinfect the same host leads to the evolution of high parasite virulence. Such models assume adaptive responses to hard selection only. We show that if soft selection is allowed to operate, low relatedness leads instead to the evolution of low virulence. With both hard and soft selection, low relatedness increases the conflict among coinfecting parasites. Although parasites can only respond to hard selection by evolving higher virulence and overexploiting their host, they can respond to soft selection by evolving other adaptations, such as interference, that prevent overexploitation. Because interference can entail a cost, the host may actually be underexploited, and virulence will decrease as a result of soft selection. Our analysis also shows that responses to soft selection can have a much stronger effect than responses to hard selection. After hard selection has raised virulence to a level that is an evolutionarily stable strategy, the population, as expected, cannot be invaded by more virulent phenotypes that respond only to hard selection. The population remains susceptible to invasion by a less virulent phenotype that responds to soft selection, however.

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Thus, hard and soft selection are not just alternatives. Rather, soft selection is expected to prevail and often thwart the evolution of virulence in parasites.

We review evidence from several parasite systems and find support for soft selection. Most of the examples involve interference mechanisms that indirectly prevent the evolution of higher virulence. We recognize that hard selection for virulence is more difficult to document, but we take our results to suggest that a kin selection model with soft selection may have general applicability.

INTRODUCTION

TWO INDIVIDUALS go hiking and they run into a bear along a trail. The bear attacks, they run, and the bear chases them down the trail. Individual *A* turns to *B* and says, "Why are we running? The bear runs faster than we." Individual *B* replies, "We don't have to run faster than the bear. I just have to run faster than you." This less offensive version of an old joke is of heuristic value because it illustrates the distinction between two forms of fitness and natural selection. If *A* or *B* were hiking alone, their fitnesses would be determined only by their ability to run from the bear. On the other hand, if *A* and *B* were hiking together, their fitnesses would be determined by their abilities to both run from the bear and to jockey with each other. Selection favors faster speeds in the first scenario, whereas in the second it may favor a balance between running and jockeying. Perhaps a more realistic situation is the relationship of gazelles and zebras with their predators. While gazelles must escape from cheetahs in a one-on-one race, zebras run as a herd when escaping from lions. Whether this explains why gazelles and cheetahs have evolved such extraordinary speeds remains to be determined, but these scenarios serve to illustrate the distinction between what has been referred to as hard and soft selection (Wallace 1970). Running speed of gazelles is a response to hard selection, whereas any jockeying behavior by a zebra is a response to soft selection.

With both hard and soft selection, fitness is always relative to the rest of the population; the distinction is what happens after the adaptation has gone to fixation (increased to a frequency of 100%). For example, the fitness of a gazelle under hard selection is first determined by the probability that the gazelle will outrun the cheetah and then by how that probability scales in relation to the average of the gazelle population. The probability of outrunning the predator is determined by a fixed or hard baseline, which in this case is the running speed of the cheetah. Thus, if a mutant gazelle adapts a faster running speed that becomes fixed in a population, the average probability of outrunning the cheetah increases after fixation. On the other hand, the fitness of a jockeying zebra under soft selection is determined by the probability that the zebra will escape predation while running with the herd. Because the probability of escape is affected by the composition of the herd, the baseline is now conditional or soft. For example, consider the jockeying behavior of hiding in the center of the herd (Hamilton 1971). If initially no zebras hide in a population, the average probability of escape equals the random probability of being drawn from the herd. If a mutant hiding zebra appears, its probability of escape will be much greater than the random expectation. However, once the hiding behavior becomes fixed and all the zebras are hiders, the probability of escape drops back to random. Because the advantage of hiding, or that of any equivalent adaptation, is dependent on the average of the population, soft selection is always frequency dependent. Hard and soft selection are not mutually exclusive, however. Even among hiders, outrunning a lion still matters and hard selection continues to operate.

The concept of hard and soft selection was first introduced by Wallace to explain why traditional models of natural selection could not account for the high frequency of allelic polymorphism observed in natural populations. If the polymorphisms were maintained by selection, the amount of selection was too much for any biologically realistic population to bear. Wallace's argument was that selection was unbearable in these situations only because the models had been cast in terms of hard selection. With hard selection, if s is the fitness difference between genotypes *A* and *B*, and a population fixed for *A* has a mean fitness of 1, then *B* is less adapted to its environment because a population with only *B* has a mean fitness of

$1 - s$. Thus, if selection operates simultaneously on many polymorphic loci, the action of hard selection on the less fit genotypes can depress mean population fitness to the point where the population does not have the fecundity to replace itself through reproduction. On the other hand, because soft selection assumes frequency dependence, the fitness of B in the presence of A may be $1 - s$, but the fitness of a population consisting only of B could have any value, including 1 or greater. Thus, soft selection can account for the persistence of polymorphisms without exacting a load because genotype B is less adapted only to the presence of genotypes A and not to the rest of its environment. However, because there are alternative explanations (Li 1997), it remains contested whether Wallace's interpretation accounts for the existence of polymorphisms.

In this article, we review evidence that suggests that hard and soft selection may actually be more useful in answering a different question: Why has it been difficult to find data that support the theoretical prediction of a negative relationship between genetic relatedness and the evolution of virulence in parasites? Conventional models have predicted that increased virulence should evolve whenever unrelated parasites coinfect the same individual host (Hamilton 1972; Bremermann and Pickering 1983; Knolle 1989; Frank 1992, 1996). We argue that this theoretical result emerges because these kin selection models have assumed the operation of hard selection only. If both hard and soft selection are allowed to operate on the parasites, the models instead predict a positive relationship between relatedness and the evolution of virulence. We present evidence that implies that low relatedness and soft selection may have prevented the increase of virulence in some biological systems. Although the idea that competition can lead to a decrease in virulence is not new, previous discussions (Holland 1986; Huang 1988; Bull 1994; Ewald 1994) have not presented the problem in terms of kin selection, or as a conflict between hard and soft selection.

Low Relatedness, High Virulence

Conventional models of parasite evolution predict a negative relationship between relatedness and virulence because the presence of unrelated parasites within a single host creates a conflict. The host is a resource; competition among the parasites favors individuals that reproduce more rapidly by exploiting the host more. The host is harmed, and virulence, defined as damage to the host, evolves to higher levels. A conflict arises because the best level of virulence for an individual parasite differs from the best level for a group of parasites that are coinfecting the same host. Relative to the group, individuals always benefit from a higher level of individual virulence. It is only when the group is highly related that individuals evolve to match the interests of the group. Relatedness effectively makes the group and the individual the same unit of selection, by reducing within-group variance while increasing between-group variance (Lewontin 1970). Without relatedness, both the group and individual parasites respond as separate units. Because the evolution of resistance on the part of the host provides a moving target for the parasites, such conventional models also assume that both parasite and host evolution have achieved a state of approximate equilibrium.

Frank (1996) has more formally modeled the effect of relatedness on virulence. As we will later expand on his analysis, we first review his model. In a population of genetically variable parasites, the fitness of a parasite of genotype a in the ith host can be expressed as

$$w_{ia} = (z_{ia})(1 - \alpha z_i) \qquad (1)$$

where α is the deleterious effect of a single parasite on the host, z_{ia} is the density (number) of parasite a in the ith host, and z_i is the total density of parasites in the same host. Thus, αz_i is a measure of virulence, and a parasite can maximize its fitness through a trade-off by increasing either z_{ia} (individual fitness) or $(1 - \alpha z_i)$ (group fitness). It should be noted that although Equation (1) closely follows the logic of the model by Frank (1996), z_i is interpreted differently here. Frank assumed that z_i equaled the average density of parasites in the ith host. We prefer our definition of z_i because it seems more reasonable that total parasite density, and not average density, should determine virulence.

The course of evolution in the parasite population described by Equation (1) can be analyzed by asking which phenotypic value of z_{ia}

is an evolutionarily stable strategy (ESS); in other words, what phenotype will produce a population that resists invasion by a genotype having any other phenotypic value (Maynard Smith and Price 1973). The ESS value (z_{ia}*) is obtained by maximizing w_{ia} with respect to z_{ia} and assuming that all genotypes have the same phenotypic value of z_{ia} (Maynard Smith 1982; Frank 1996). Thus, if n is the average size of the coinfection group, $z_i = nz_{ia}$. By taking the partial derivative of Equation (1) and setting it equal to zero,

$$\delta w_{ia} / \delta z_{ia} = 1 - \alpha z_i + z_{ia}(-\alpha \delta z_i / \delta z_{ia})$$

$$0 = 1 - \alpha n z_{ia}^* + z_{ia}^*(-\alpha \delta z_i / \delta z_{ia})$$

$$\alpha z_{ia}^* = 1 / (n + \delta z_i / \delta z_{ia}) \quad (2)$$

Because genetic relatedness can be manifested phenotypically by the resemblance between relatives, the average value of z_i should be similar to z_{ia} in the ith host when genetically related parasites coinfect the same host (Frank 1996). Thus, the coefficient of genetic relatedness r experienced by genotype a is measured by the regression of z_i / n onto z_{ia}, or

$$r = \delta(z_i / n) / \delta(z_{ia}) \quad (3)$$

$$= (1 / n)\delta z_i / \delta z_{ia} \quad (4)$$

By combining and rearranging Equations (2) and (4),

$$z_{ia}^* = 1 / [\alpha n(r + 1)] \quad (5)$$

Since $z_i = nz_{ia}$*, and virulence is defined as αz_i, the ESS level of virulence is

$$\alpha n z_{ia}^* = 1 / (r + 1) \quad (6)$$

If the overall parasite population (summed over all coinfection groups) is large, and coinfection is random, then $r = 1 / n$ and

$$\alpha n z_{ia}^* = n / (n + 1) \quad (7)$$

Thus, the effect of relatedness on virulence in conventional models of parasite evolution is summarized by Equations (6) and (7). The minimum value of virulence that is an ESS equals 1/2, which is achieved when $r = 1$ or $n = 1$. This value is the optimal level of virulence for the group. If relatedness is decreased or group size is increased, individual selection begins to oppose group selection, so the ESS level of virulence increases above the group optimum. Because of the difference in assumptions (see above), Equation (6) differs from Frank's (1996) solution for the ESS level of virulence. Frank obtained the result $\alpha z_{ia}^* = 1 - r$, which gives the same qualitative relationship between virulence and r. However, we find our result to be more realistic because virulence clearly should not evolve to zero with increasing r. If $n = 1$, the parasite and its progeny must still achieve a density $z_{ia}^* > 0$ in order to be transmitted.

Support for the Conventional Model

Most of the support for a kin selection model of virulence has been either indirect or based on the appeal of its explanatory power. Frank (1996) carefully and correctly pointed out that many of the previous models and evidence for increased virulence may have confounded the effects of horizontal and vertical transmission with the effects of relatedness among the transmission groups. For example, when Herre (1993) measured the degree of virulence exhibited by nematodes infecting fig wasps, he observed an increased virulence in those wasp species that typically colonized a fig with multiple foundresses. Herre's view was that virulence was higher with multiple foundresses because the nematodes could be transmitted horizontally to different wasps within a fig. Frank reinterpreted Herre's result and suggested instead that because multiple foundresses brought together unrelated nematodes, the increased virulence could be as easily or even more appropriately explained by a difference in the relatedness. Frank's position was that transmission mode (horizontal versus vertical) is often inversely correlated with relatedness, but the correlation can be broken and what ultimately matters is relatedness. A case in point is the transmission of cytoplasmic organelles during the fusion of gametes in sexually reproducing organisms (Hurst and Hamilton 1992; Frank 1996). Although transmission is vertical, the fusion allows unrelated organelles to mix. Conventional kin selection models predict an increase in the intracellular density (and hence virulence) of competing organelles, despite any damage that could be done to the cell and the resident chromosomal elements. The observation that cytoplasmic elements are so often transmitted by one parent only—presumably

by a transmission process controlled by the larger and more numerous chromosomal elements—stands as one of the stronger arguments for the importance of relatedness over mode of transmission in determining the evolution of parasite virulence.

Given the explanatory power of a kin selection model for the evolution of virulence, however, the lack of more extensive tests of the theory is disappointing. Although studies have clearly demonstrated the evolution of virulence (or avirulence) and the importance of transmission (Bull et al. 1991; Herre 1993; Fenner and Kerr 1994; Ebert and Mangin 1997; Turner et al. 1998), few have actually examined the direct effects of relatedness or coinfection rates. One exception is Read et al. (1999), who recently initiated a more systematic examination of the theory. They first surveyed data from several studies of *Plasmodium falciparum*, a malarial protozoan of humans, and compared the number of clones per infection in people with asymptomatic infections, mild malaria, and severe malaria. In most studies, the number of clones per infection was unrelated to the severity of the infection; two studies found a higher number of clones associated with less severe infections, and only one study showed a positive association between number of clones per infection and the severity predicted by the kin selection models. Because field surveys of parasitism are notoriously difficult to interpret, Read et al. also experimented with *P. chabaudi* infections in mice. Although virulence was higher (more anemia and 30% more weight loss of the host mice) in infections started with mixed clones of *Plasmodium*, total parasite densities were not. Thus, their first result is consistent with predictions of kin selection models, but the second is not. Read et al. suggest that the increased virulence may be due to an increased cost to the host in combating a mixed infection. Clearly, additional and unknown interactions may be operating, but these parasites did not behave as predicted by conventional kin selection models for virulence.

In summary, although some correlational evidence is supportive, the predicted evolutionary relationship between relatedness and virulence has not emerged in recent experiments designed specifically to test the proposition. Whether this points to a shortcoming of the theory or whether additional information on any of the above examples could uncover more favorable evidence, progress in evaluating the theory is limited by the difficulty of manipulative experiments. More detailed and more reliable information from experiments could allow a better evaluation of the models, their assumptions, and predictions. The amenability of viral systems to experimental evolution has recently allowed for just such an analysis. These studies show that the kin selection models are correct in identifying the conflict. Because they assume only hard selection, however, they may not predict the correct evolutionary response to the conflict. The biological reality may be that adaptations through soft selection can evolve more readily and prevent the evolution of virulence.

Defective and Defecting Viruses

Viruses, like all parasites, depend on a host for replication. Thus, if two or more viruses coinfect the same host, the same trade-offs between density, virulence, and relatedness represented by Equation (1) also apply. Taking advantage of the short generation time of viruses, Turner and Chao (1998, 1999) tested the effect of relatedness in evolving cultures of the RNA bacteriophage $\phi 6$, which infects the bacterium *Pseudomonas phaseolicola*. Relatedness was controlled by diluting the phage population and thus manipulating the multiplicity of infection (the number of infecting phage to host cells). At low multiplicity, each phage infects a host cell alone, so relatedness within a cell is high. On the other hand, relatedness is lower at high multiplicity because two or more phage infect the same cell. After 250 generations of evolution, fitness (density relative to a reference $\phi 6$ phage) was tested under the two treatments of high and low multiplicity. Contrary to the predictions of conventional kin selection models of virulence, the low-multiplicity lineages had evolved higher fitness whereas the high-multiplicity ones had evolved lower fitness. Also, the fitness that the phage evolved at high multiplicities was frequency dependent. When the high-multiplicity phage was rare relative to the reference phage, its fitness was high. When it was common, its fitness was low.

Because the reference $\phi 6$ was the ancestral phage used to start these evolution experiments, the reported fitness values represent actual evolutionary changes. As a result, the frequency-dependent outcome is important because it reconstructs the evolutionary history of the phage evolved at high multiplicity (hereafter the evolved phage, unless indicated otherwise). The low frequency corresponds to when the evolved phage was rare and first appeared as a mutation, whereas the high frequency corresponds to its approach to fixation. Furthermore, when the evolved phage was rare, its fitness was determined primarily by its membership in "mixed" coinfection groups (ones containing both the evolved and the ancestor phage). On the other hand, still considering only when the evolved phage was rare, the fitness of the ancestor phage was determined primarily by its membership in "pure" coinfection groups (ones containing only ancestor phage). By the same argument, when the evolved phage became common, the fitness of the evolved and ancestor phage were determined primarily by their memberships in, respectively, pure (evolved phage only) and mixed coinfection groups. Thus it is possible to represent the frequency-dependent result as a 2×2 matrix in which the entries represent fitness values for each phage in mixed and pure coinfection groups.

A fitness matrix (Figure 1) estimated from the frequency-dependent result reconstructs the following scenario. When rare, the fitness of the evolved phage was 1.99 in mixed infections and the fitness of the ancestor was 1.00 in pure infections (ancestor only). Thus, the evolved phage was able to increase in frequency and invade the population of ancestral phage. However, as the evolved phage increased in frequency and found more of itself in pure infections (evolved phage only), its fitness began to drop towards 0.83. At the same time, the ancestral phage decreased in frequency. When it became rare, it found itself primarily in mixed coinfection groups in which its fitness was 0.65, a value less than 0.83, and went extinct. The final fitness of the evolved population was 0.83, which is lower than the ancestral fitness of 1.00.

Why should a phage evolve a lower fitness? Realizing that their fitness matrix conformed to a payoff matrix in game theory (Maynard Smith 1982), and that the frequency dependence generated by the matrix introduced soft selection, Turner and Chao (1998, 1999) interpreted their results by combining these two perspectives. They proposed that the lower fitness evolved because the conflict between unrelated viruses within a coinfection group led to soft selection rather than to hard selection. The evolved phage had a higher fitness in mixed infections, a result of adaptation through soft selection. In the process, however, the phage also evolved a lower fitness in pure infections. Thus, adapting under soft selection entails a cost that is paid as a decreased ability in exploiting the host.

We do not know in what characteristics the phage has adapted, or why there is a cost, but studies on other viruses suggest possibilities. For example, because a viral genome serves both as a template for replication and transcription (protein synthesis), a virus will be selected to balance its allocation to these two functions. However, if coinfection of the same host cell by many viruses is common, one virus can evolve to allocate more to replication and come to rely on other viruses to produce the required proteins. The application of game theory to viral evolution is thus appropriate; viruses that specialize in protein production are effectively cooperators, and ones that specialize in replication are defectors. Defector strategies in viruses are evidenced by the frequent evolution of defective interfering (DI) RNAs (Holland 1986; Chao 1994). The similarity between the name "defective" and the strategy "defector" is purely coincidental. DI RNAs are defective because they are viral RNAs that have lost all or most of their protein-coding sequences and are unable to replicate in the absence of coinfecting complete viruses. Because complete viruses provide all of the required proteins, DI RNAs are encapsidated and packaged into viral particles. However, DI RNAs are also defectors because they have forgone protein production while evolving mechanisms to sequester a larger share of the resources in the presence of coinfecting complete viruses. Some DI RNAs replicate more quickly by virtue of their smaller size. Others possess extra sequences recognized by encapsidation and replication enzymes, and are thus preferentially processed (Figure 2).

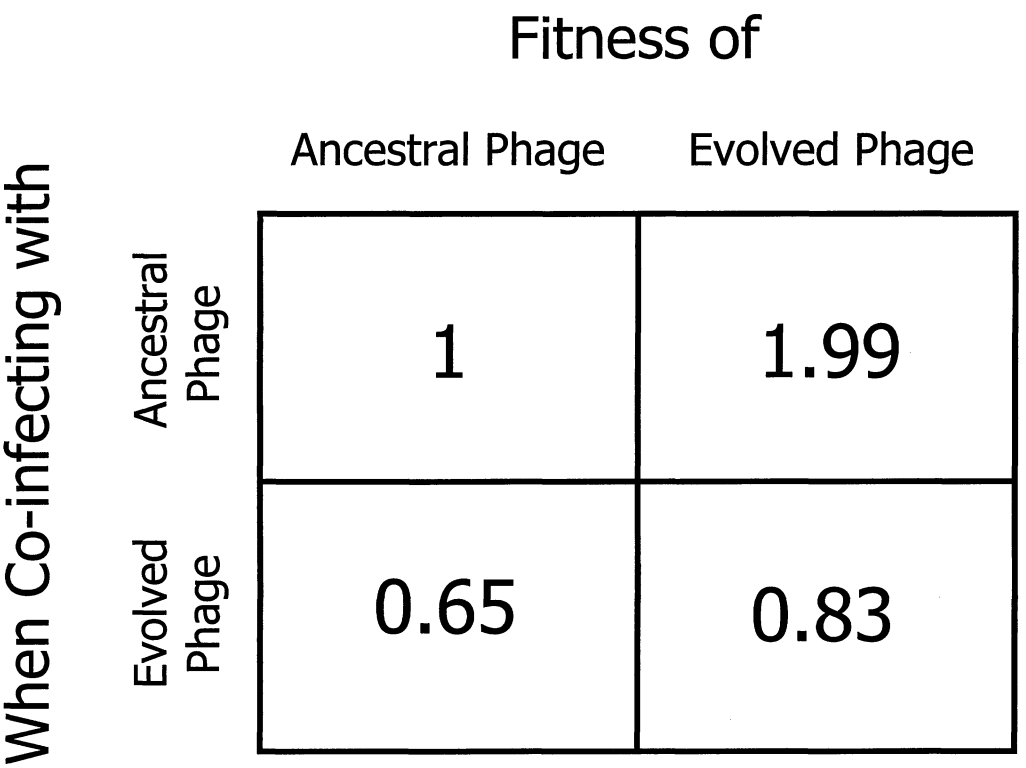

FIGURE 1. FITNESS PAYOFF TO COINFECTING ANCESTRAL AND EVOLVED PHAGE.
The main diagonal of the matrix corresponds to fitness for each phage in pure coinfections and the off diagonal to that in mixed coinfections. When a phage is rare in frequency, its fitness is primarily determined by its membership in mixed coinfections; when it is common, its fitness is primarily determined by its membership in pure coinfections. Selection for the evolved phage is frequency dependent because the phage has a fitness of 1.99 when rare and 0.83 when approaching 100%. Thus, the phage evolved in response to soft selection. Selection for the ancestral phage is also frequency dependent, but the phage goes extinct because it has the lowest fitness when rare. Because the system evolves from an ancestral fitness of 1.0 to a final fitness of 0.83, the resulting matrix corresponds to the payoffs of the Prisoner's Dilemma.
DI RNAs do not evolve into the Prisoner's Dilemma because they are unable to replicate in a pure coinfection (see Figure 2). Thus, their fitness entry in the lower right quadrant is zero instead of 0.83 and their evolution leads to a stable polymorphism in which they coexist with the ancestral virus.
Figure adapted from Turner and Chao (1999).

Thus, DI RNAs interfere with (hence their second name) or reduce the fitness of complete viruses by usurping resources. They gain by this interference; that constitutes their adaptation under soft selection. However, being smaller, having fewer genes, or having extra encapsidation and replication signals can be deleterious in pure infections without complete viruses; that constitutes the cost for adapting under soft selection.

LOW RELATEDNESS, LOW VIRULENCE

The outcome of evolving a lower final fitness by maximizing individual fitness at the expense of group fitness is often referred to as the "Tragedy of the Commons" in ecology or as the "Prisoner's Dilemma" in game theory (Axelrod and Hamilton 1981; Frank 1996). In the above $\phi 6$ example, it is soft selection that traps the phage in the Prisoner's Dilemma, but the same problem can arise with hard se-

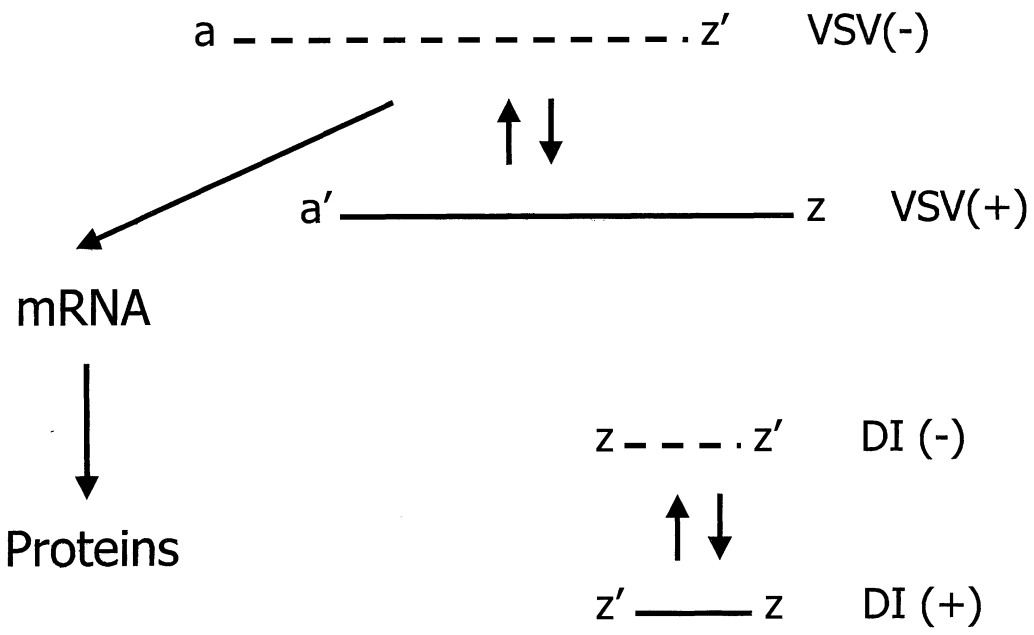

FIGURE 2. SOFT SELECTION IN VESICULAR STOMATITIS VIRUS.

Vesicular Stomatitis Virus (VSV) is a single-stranded RNA virus. The infectious virus is designated as the negative strand genome, VSV (−), because it is the template for transcribing the messenger RNA (mRNA), which is by definition positive stranded. However, VSV (−) also replicates by serving as the template for the complementary positive-strand genome, VSV (+), which in turn is the template for making more negative-strand genome. The 3′ terminus of VSV (−) contains an initiation site (**a**) for both transcription and replication, whereas the 3′ terminus of VSV (+) has a site (**z**) for only replication. The complementary sequences of **a** and **z** on the opposite strands are designated as **a′** and **z′**. Such VSV genomes are functionally complete because they can replicate when they are alone in infecting a cell.

Defective interfering (DI) RNAs of VSV are parasites produced by soft selection. One type of such DI RNA is illustrated here. It is shorter and lacks any protein coding sequences. More importantly, it has a **z** site at the 3′ terminus of both its negative and positive strands. Whereas VSV (−) allocates time for transcription, DI (−) allocates none and it is effectively equivalent to DI (+). Thus, although the DI RNA relies on complete viruses to provide proteins, including the polymerase needed for replication, it has a higher replicative fitness than the VSV genome. Because of the reliance on complete viruses, the fitness of the DI RNA is frequency dependent and the duplication of the **z** site is an adaptation to soft selection. As this DI RNA is unable to replicate when it is alone in infecting a cell, it well illustrates the cost of adapting to soft selection.

Figure modified from Holland (1986).

lection in parasite evolution. With hard selection, the conflict between coinfecting parasites leads to the evolution of higher parasite density. As a result, the host is overexploited and virulence increases. On the other hand, with soft selection, the resulting adaptations do not lead to an increase in parasite density and overexploitation is avoided. However, if the viral results above serve as an example, the adaptations will incur a cost and the host is actually underexploited. Thus, with regards to the evolution of virulence in parasites, the "tragedy" of hard selection is the overexploitation of the host, whereas with soft selection it is the underexploitation.

The effect of relatedness on hard and soft selection models of parasite virulence is therefore essentially determined by its effect on the Prisoner's Dilemma (or the Tragedy of the Commons). One mechanism to escape the Prison-

er's Dilemma is to play an iterated game with no predictable limit (Axelrod and Hamilton 1981), but kin selection is another (Hamilton 1972; Nowak and May 1994). Increased relatedness favors cooperation by discriminating against kin groups of defectors. In turn, cooperation allows the parasites to exploit the host more optimally (neither over nor under), in which case hard and soft selection models lead to opposite predictions. With soft selection, relatedness can increase virulence when it reduces the underexploitation of the host. The latter outcome was in fact also observed in the above ϕ6 studies (Turner and Chao 1998). Under low multiplicity—a treatment that promotes clonal reproduction (see above)—phage evolved higher reproductive densities.

Given that hard and soft selection can make opposite predictions, however, which one is more likely to determine the outcome of evolution in a group of coinfecting parasites? The problem can be approached theoretically, but it is difficult to combine the effects of soft selection into Equations (6) and (7). The reason is that soft selection introduces frequency-dependent selection; ESS outcomes then depend on how parasites with varying rates of defection and cooperation will interact with varying frequencies of each other. However, it is possible to gain some insights by examining the invasion conditions.

Let the ESS values derived above (Equations (1–7)) for hard selection serve as a baseline for comparison. The fitness of the ESS genotype a under hard selection is

$$w_{ia}^* = (z_{ia})(1 - \alpha n z_{ia}^*) \quad (8)$$

If only hard selection is possible, then the invasion conditions can be examined by considering a mutant b that deviates from z_{ia}^* by a magnitude e. Thus, the density of mutant b is $z_{ia}^* \pm e$ and its fitness becomes

$$w_{ib} = (z_{ia}^* \pm e)(1 - \alpha((n - 1)z_{ia}^* + z_{ia} \pm e)) \quad (9)$$

The values of n and z_{ia}^* affect w_{ib} because genotype b would be initially rare and find itself primarily in mixed coinfections with $n - 1$ parasites of genotype a. Rearranging Equation (9) and substituting in Equation (7),

$$w_{ib} = (z_{ia}^*)(1 - \alpha n z_{ia}^*) - \alpha e^2 \quad (10)$$

in which case, the sign of e does not affect the magnitude of w_{ib} because the term is squared. As $-\alpha e^2$ is always negative, $w_{ib} < w_{ia}$ (cf. Equations (8) and (10)), and this result simply confirms the earlier analysis that z_{ia}^* is an ESS under hard selection.

However, z_{ia}^* no longer remains an ESS if adaptation through soft selection is possible. Taking the above ϕ6 results as a guide, let a novel genotype c take resources from genotype a. As a result, z_{ia}^* will be depressed and the density of genotype c will be elevated above z_{ia}^*. If $z_{ic} = z_{ia}^* + e$ and x is the amount by which z_{ia}^* is depressed, the fitness of genotype c when it appears as a rare mutant is

$$w_{ic} = (z_{ia}^* + e)(1 - \alpha((n - 1)z_{ia}^* - x + z_{ia}^* + e)) \quad (11)$$

$$w_{ic} = (z_{ia}^*)(1 - \alpha n z_{ia}^*) + \alpha x z_{ia}^* - \alpha e(e - x) \quad (12)$$

If $e \leq x$, then $w_{ic} > w_{ia}^*$ and genotype c is able to invade a population of genotype a (cf. Equation (11)). If $e > x$, then genotype c is able to invade only if the last two terms of Equation (12) are greater than zero, or

$$0 < \alpha x z_{ia}^* - \alpha e(e - x) \quad (13)$$

$$(e / x)(e - x) < z_{ia}^* \quad (14)$$

The invasion conditions are now restricted, because if genotype c overly elevates e, it hurts itself by becoming too virulent. For Equation (14) to be true, the value of e must be reduced, either as a ratio or a difference with respect to x. Nonetheless, whereas genotype a could not be displaced under hard selection, a novel genotype c can invade under soft selection. Then virulence evolves away from the previous ESS value of $\alpha n z_{ia}^* = n / (n + 1)$ (see Equation (7)).

The invasion by genotype c can have an initial and a long-term effect on the virulence of the parasite. The initial effect depends on the relationship between e and x. If $e < x$, virulence decreases as the genotype increases in frequency because $e - x < 0$. If $e > x$, then $e - x > 0$, and soft selection actually increases virulence as genotype c becomes more common. Whichever the case, the long-term effect on virulence will depend less on e and x—which are parameters of mixed coinfection groups—and depend more on the properties of pure coinfection groups containing only genotype c.

As genotype c increases to fixation (or a high frequency), it will find itself only (or primarily) in pure coinfection groups. If the ϕ6 results are a guide, a mutant such as genotype c should be constrained by a trade-off between sequestering and performance in pure coinfections. Thus, without other genotypes to provide gene products, the density of genotype c will drop as it increases in frequency, and the long-term evolution of virulence is downwards.

This analysis reveals two important results. First, as suggested earlier, the effect of soft selection on the relationship between relatedness and virulence is the opposite of hard selection. Second, the invasion by genotype c when genotype b was unable to increase is important because it shows that a response to soft selection is possible even when a response to hard selection is not. Therefore, hard and soft selection are not simply alternatives. It is more likely that soft selection will prevail and be the primary force directing the evolution of parasites within coinfection groups. We do not know whether real organisms will show as plastic a response to selection as assumed by the models clearly, but the immediate value of these results is to provide alternative and potentially testable hypotheses. For example, they may explain why virulence and coinfection are not correlated in the *P. falciparum* populations reviewed by Read et al. (1999). However, the lack of a strong (and negative) correlation does imply that if interference is occurring, either the difference $e - x$ or the trade-off cost of interference is small (see above). Thus, it may be instructive to examine whether there is interference among clones. If there is, do clones that are good at interfering pay a cost in pure coinfections? *Plasmodium falciparum* may not be the only system that could be examined from the perspective of soft selection. Viruses (in addition to ϕ6) and bacteria are obvious candidates. Ewald (1994) has suggested that the decrease in toxin production (and virulence) often observed in the course of a *Vibrio cholera* infection may be due to intrahost competition among coinfecting strains of the bacterium. Bacterial plasmids and digenetic trematodes are two other candidates, which we review next.

Worm-Eating Worms

Digenetic trematodes are parasites that use snails as intermediate hosts. In the typical life cycle, the trematode infects a snail as a motile miracidium and metamorphizes into a sac-like sporocyst. The sporocyst produces embryos that develop into mobile rediae, which, unlike the sporocyst, possess a mouth and gut. Rediae feed on host tissues, usually the gonads, and produce embryos that develop into cercaria, which then move out of the snail to infect a second intermediate host such as a fish. Many trematode species may infect a population of snails, and infection rates can be high. One study of the horn snail (*Cerithidea californica*) documented seventeen species of trematode infecting 68.2% of the host population (reviewed in Kuris 1990). Despite the high infection rate, however, snails coinfected with more than one trematode species were less common than expected by chance.

This pattern of negative association among trematode species is attributed to antagonistic interactions among the species (Kuris 1990; Sousa 1990). Individual trematode species are able to eliminate a subset of coinfecting competitors, and a guild can be ranked into a dominance hierarchy based on the ability of each species to displace the others. In some trematodes, the rediae eliminate competitors by consuming the rediae and sporocysts of a coinfecting species. Other species do not produce rediae, and the elimination of competitors is achieved by the sporocysts. The mechanism of elimination is unknown, but it cannot be mediated by consumption because sporocysts lack mouths.

The evolution of mechanisms to eliminate coinfecting competitors is an adaptive response to soft selection. Therefore, we predict a negative relationship between coinfection and virulence in trematodes. With consumption by rediae, $e < x$ because energetic transfer is imperfect. Therefore, the final biomass of trematodes should be less after consumption than before. With elimination by sporocysts, the requirement $e < x$ should be even more likely satisfied because there is no consumption and caloric gain to the sporocysts. Thus, by eliminating or consuming their competitors, the dominant trematodes effectively remove any advantage for increasing virulence (such as con-

suming additional host tissues). Furthermore, if the mechanism of elimination incurs a cost in pure infections, as sequestering does in viruses (see above), then we expect that the evolution of such interference mechanisms should actually decrease both parasite density and virulence after the trait has gone to fixation.

War of the Plasmids

The defining distinction between bacterial chromosomes and the circular DNA molecules known as plasmids is that the latter are not essential for the bacterium under many conditions. Thus it is agreed that the bacterium is the chromosome and, because plasmids autonomously replicate within a cell, plasmids use the bacterium as a host. However, the exact evolutionary relationship between chromosomes and plasmids is debated.

Plasmids can provide beneficial genes in the form of antibiotic resistance or metabolism of novel substrates (Timmis and Puhler 1979). Their relationship to their host, viewed simply from this perspective, is clearly mutualistic. The relationship between plasmids and bacteria is complicated through the ability of plasmids to replicate by two alternative mechanisms, however. They can replicate in step with the bacterial chromosome and be transmitted vertically, but they are also able to replicate independent of the chromosome and transfer themselves horizontally from their host cell to a neighboring cell by a process known as conjugation (Willetts 1979).

Conjugation requires the coordinated synthesis of pili, transfer initialization, and plasmid replication. Pili are outer cellular appendages necessary for completing conjugation, but their exact function is not known. However, it is known that conjugation is costly to the host cell (Levin 1980; Turner et al. 1998). Thus, the cost of horizontal transmission turns plasmids into parasites. Because maximizing conjugation increases virulence and decreases the chances of vertical transmission, plasmid fitness conforms to Equation (1). How then do plasmids deal with the presence of unrelated plasmids coinfecting in the same bacterial cell? A response to hard selection should increase both conjugation rates and virulence. Many of the adaptive responses of plasmids have been characterized and found not to increase virulence. Detailed studies have instead revealed mechanisms of interference that are characteristic of soft selection.

Plasmids can be categorized by their mechanisms of surface exclusion and incompatibility (Datta 1979). Although the traditional classification is primarily by incompatibility, surface exclusion is an alternative that is also appropriate in the context of the present analysis. Plasmids that have the same surface exclusion are unable to transfer into a cell that already contains a plasmid of that exclusion group. Thus, although surface exclusion is not an adaptive response to the direct presence of unrelated plasmids in the same host cell, it is likely a response to the possibility that an unrelated plasmid may attempt to enter the cell.

Incompatibility, on the other hand, operates after two plasmids have entered the same cell. Whether two plasmids belong to the same incompatibility group depends on whether their regulation is functionally similar (Novick 1987). If they are sufficiently similar, the two plasmids will respond equally to regulation in the same cell and no one plasmid can change its intracellular frequency by responding differently. As a result, if the frequency of one plasmid decreases by chance, it remains at the lower value. As the frequency can also drift to zero by chance, the rarer plasmid is now more vulnerable to segregation (loss from the cell). Thus, incompatibility is the consequence of the fact that shared regulation prevents the plasmids from recovering if their frequencies decrease. If the plasmids are at the same frequency, their likelihood of segregational loss is equal. However, because an invading plasmid begins as one copy after it is transferred into a cell, it will always be rarer then the resident plasmid and be at a segregational disadvantage. Thus, incompatibility, like surface exclusion, benefits the resident plasmid, but, unlike exclusion, it terminates rather than prevents coinfection. However, one common consequence of both incompatibility and surface exclusion is the prevention of the escalation of virulence between plasmids that coinfect or could coinfect the same host cell.

If the replication of two plasmids have sufficiently different mechanisms of regulation, they can become compatible and are classified into different incompatibility groups. They will now

be able to coexist in the same host cell, but from an evolutionary perspective, the stability is short-termed and not an ESS. They are no longer regulated by the same mechanism, but they may still share gene products and the same host cell. They will each always benefit by responding to hard selection and evolving higher rates of replication and transfer. As both replication and transfer can be costly to the host, virulence should increase. Despite this prediction, however, what is again observed in plasmids is the effects of soft selection preventing a response to hard selection. In many plasmids, specific mechanisms have evolved that directly interfere with the transfer of plasmids coinfecting the same cell. These mechanisms are described as fertility inhibition.

Many different mechanisms of fertility inhibition are known. In the conjugative plasmid RP1, which belongs to the IncP incompatibility group, the FiwA locus inhibits the transfer of plasmids in the IncW group (Yusoff and Stanisich 1984; Fong and Stanisich 1989). In turn, RP1 is inhibited by the FipA and PifC loci of pKM101 (IncN) and F (IncFI) plasmids, respectively (Winans and Walker 1985). The interference mechanism of FipA and PifC has been well characterized and it is known to act on the initialization of transfer (Figure 3). The transfer of RP1 requires the initial attachment of the plasmid to the DNA transport complex, and the process is mediated by a coupling protein known as TraG (Cabezon et al. 1997). The gene products of both FipA and PifC act by directly interfering with TraG (Santini and Stanisich 1998). A second known mechanism of fertility inhibition is determined by FiwB, a locus that is also found on RP1 (Yusoff and Stanisich 1984; Fong and Stanisich 1989). The details of the mechanism have not been worked out, but FiwB appears to directly inhibit the synthesis of pili in IncW plasmids.

Without knowing the ancestral state, it is not possible to determine whether plasmids have evolved a higher level of virulence in response to coinfection. However, it is clear from these examples that the evolutionary response in bacterial plasmids has been strong in regards to soft selection. If there is a cost to interference by either surface exclusion, incompatibility, or fertility inhibition, the ability of an interfering plasmid to exploit its host cell may be compromised. A decreased ability should again lead to lower virulence when competing plasmids have been eliminated from the host cell or the interfering plasmid has gone to fixation in the population. Thus, the evolution of plasmids provides additional support for the predictions of our soft selection models of virulence.

Discussion

Conventional models predict that low genetic relatedness among parasites coinfecting the same host will lead to the evolution of high parasite virulence. We have shown that this traditional result emerges because these models allow adaptive responses only to hard selection. We find that if responses to soft selection are allowed, low relatedness leads instead to lower virulence. With both hard and soft selection, decreased relatedness intensifies the conflict among parasites coinfecting the same host. However, with hard selection the parasites can respond only by evolving higher densities and overexploiting the host; with soft selection they can evolve adaptations, such as interference, that prevent overexploitation. Because interference can entail a cost, the host may actually be underexploited, which amounts to a decrease in virulence.

We also find that soft selection can override hard selection. Once parasite densities (and virulence) have evolved to an ESS level in response to hard selection, the population, as expected, cannot be invaded by more virulent phenotypes that respond only to hard selection. However, the population is vulnerable to invasion by less virulent phenotypes that respond to soft selection. Thus, hard and soft selection may not just be alternatives. Rather, our analysis suggests that soft selection is expected to prevail and more often govern the evolution of virulence in parasites.

Our review of several parasite systems provides support for the importance of soft selection and the evolution of virulence. Admittedly, care must still be taken in assessing these results because soft selection is inherently easier to document than hard selection. Whereas adaptation through soft selection can be identified by simply documenting the mechanism, adaptations to hard selection require knowledge of both the ancestral state and the level of coinfection. For example, field studies of

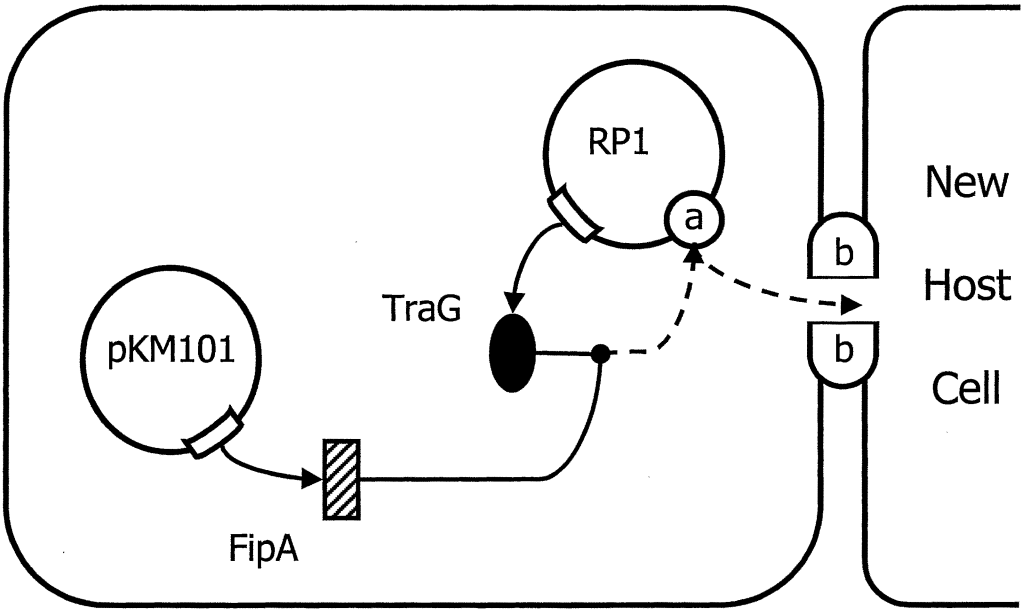

FIGURE 3. THWARTING VIRULENCE IN COMPATIBLE BACTERIAL PLASMIDS.
RP1 and pKM101 are two compatible plasmids that can inhabit the same bacterial cell. Both are conjugative and able to promote their own transfer to a new host cell. To initiate conjugation, RP1 forms a relaxasome (a), which is required to nick and, later on, to unwind the plasmid DNA. The protein TraG, which is also encoded by RP1, couples the relaxosome to the DNA transport complex (b) to complete the transfer of plasmid DNA. However, plasmid pKM101 inhibits the transfer of RP1 by producing the FipA protein, which interferes with the coupling activity of TraG. Thus, any advantage for RP1 to increase virulence and transfer rates, such as by synthesizing more plasmids, relaxosomes, transport complexes, or TraG, is thwarted by FipA.

the myxoma virus have clearly documented the evolution of decreased virulence (Fenner and Kerr 1994). Because the ancestral virus had been saved in the laboratory, it could be used as a control to demonstrate the change in virulence. However, without additional information on the level of coinfection in the field, hard selection cannot be reliably invoked to explain changes in virulence. An additional caveat is that the identification of low coinfection rates does not alone rule out soft selection. As surface exclusion and incompatibility in plasmids show, soft selection can produce mechanisms that prevent high coinfection rates.

One may question whether all of our examples of soft selection are truly cases of adaptation. For example, plasmid incompatibility could also have arisen indirectly, simply as a side consequence of regulation and not as an evolved response to any direct selection. To know whether it is an adaptation, one would need to compare the strength of the incompatibility in plasmids that have evolved with and without the presence of coinfecting plasmids. If incompatibility is stronger in the presence of coinfection, the increase represents the adaptive value of incompatibility as a mechanism of interference. The incompatibility level achieved by the plasmid when evolved alone represents the amount that can be attributed to a side consequence of regulation. It is very unlikely that none of the described mechanisms are adaptations, however, in which case soft selection has likely operated and prevented the evolution of virulence by hard selection in a variety of biological systems.

The proposal that soft selection mainly determines the evolution of virulence also suggests alternative interpretations for known patterns of virulence. For example, one interpretation—

the proposal that uniparental inheritance evolved as a mechanism imposed by chromosomes to prevent the evolution of virulence on the part of cytoplasmic elements—relies on the conventional models of hard selection (see above). If soft selection is overriding hard selection, virulence should not be increased by biparental inheritance. Instead, we expect that soft selection and biparental inheritance should lead to the evolution of interference. As there may be a cost to interference, the density and virulence of the cytoplasmic elements should decrease. In this case, however, decreasing the density of cytoplasmic elements is not beneficial for the host. The elements are not parasites, but contributing symbionts. Reducing their density only reduces the benefits of their mutualistic functions. Thus, uniparental inheritance may have evolved to prevent a conflict between cytoplasmic elements, but the outcome that was obviated may not have been increased virulence, but rather a reduction in mutualistic functions.

Adaptations through soft selection could also have a strong limiting effect on the progress of infections by parasites that are able to complete many cycles of replication within a host. With multiple generations, parasite evolution will ensue within the lifetime of the host. Even if the infecting parasites were initially related, they will become less so with time and mutations. If this decrease in relatedness modifies adaptation through soft selection, the virulence of the parasites could decrease. Such an attenuation provides a built-in mechanism of negative feedback. Successful infections would have the highest parasite densities, which in turn leads to stronger soft selection and attenuation. Viruses are an obvious candidate for such attenuation processes (Turner and Chao 1998, 1999). DI RNAs could also attenuate by interfering with the effectiveness of complete viruses (Holland 1986; Huang 1988). The long-term monitoring of infections within a single host would greatly help to assess the reality of such a process.

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