Escape from Prisoner's Dilemma in RNA Phage Φ6

Paul E. Turner[1,*] **and Lin Chao**[2,†]

1. Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut 06520;
2. Department of Biology, University of California, San Diego, California 92093

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ABSTRACT: We previously examined competitive interactions among viruses by allowing the RNA phage Φ6 to evolve at high and low multiplicities of infection (ratio of infecting viruses to bacterial cells). Derived high-multiplicity phages were competitively advantaged relative to their ancestors during coinfection, but their fixation caused population fitness to decline. These data conform to the evolution of lowered fitness in a population of defectors, as expected from the Prisoner's Dilemma of game theory. However, the generality of this result is unknown; the evolution of viruses at other multiplicities may alter the fitness payoffs associated with conflicting strategies of cooperation and defection. Here we examine the change in matrix variables by propagating the ancestor under strictly clonal conditions, allowing cooperation the chance to evolve. In competitions involving derived cooperators and their selfish counterparts, our data reveal a new outcome where the two strategies are predicted to coexist in a mixed polymorphism. Thus, we demonstrate that the payoff matrix is not a constant in Φ6. Rather, clonal selection allows viruses the opportunity to escape the Prisoner's Dilemma. We discuss mechanisms that may afford selfish genotypes an advantage during intrahost competition and the relevance in our system for alternative ecological interactions among viruses.

Keywords: coinfection, evolution, frequency dependence, game theory, polymorphism, virus.

The fitness of a particular phenotype often depends on the frequency of its own and other phenotypes in the population. Evolutionary game theory is a method whereby evolution can be analyzed when fitnesses are frequency dependent (Maynard Smith 1982). Suppose that a population contains two phenotypes and that these phenotypes are referred to as strategies. Before reproduction, an individual engages in a pairwise interaction with a random partner. An individual's fitness consists of a constant value plus a payoff; this payoff is the change in fitness resulting from the interaction. After the interaction, individuals reproduce their kind and die, and the numbers of offspring produced are proportional to their fitnesses. Of interest is whether one phenotype in the population is an evolutionarily stable strategy, such that the strategy prevents invasion by mutants playing any other strategy. However, if no single strategy is able to resist invaders, a polymorphic equilibrium with both strategies results.

The biological literature has addressed the temptation for individuals to defect (cheat) for personal reward, in lieu of cooperative behavior that promotes the common good (e.g., Axelrod 1985; Dugatkin 1997). Game theory allows a contest between cooperators and defectors to be described by a 2×2 payoff matrix (fig. 1*A*). When two cooperators interact, their individual fitness is 1. When a cooperator and a defector are paired, the cooperator is exploited and its fitness is decreased by s_1, whereas the defector benefits and its fitness is increased by s_2. When two defectors interact, they suffer from not having anyone to exploit, and they pay a cost c. If $(1 - c) < (1 - s_1)$, a polymorphic equilibrium occurs. If $(1 - c) > (1 - s_1)$, the population evolves to be entirely defectors. The latter is termed the Prisoner's Dilemma, and it is evolutionarily paradoxical because a population composed of defectors has a lower fitness than a population containing only cooperators.

Viruses are obligate intracellular parasites that usurp control of the host cell's biomolecular machinery to complete their life cycle. During reproduction, viruses manufacture products that diffuse within the cell and prevent an individual virus from having exclusive access to its own gene products. This creates a conflict of interest whenever multiple viruses infect a single host (Nee and Maynard Smith 1990; Nee 2000; Brown 2001), allowing for the strategies of cooperation and defection. A virus that makes large (excess) amounts of product benefits other coinfecting genotypes, and the virus can be defined as a cooperator. In contrast, a virus that synthesizes less but

* Corresponding author; email: paul.turner@yale.edu.

† E-mail: LChao@biomail.ucsd.edu.

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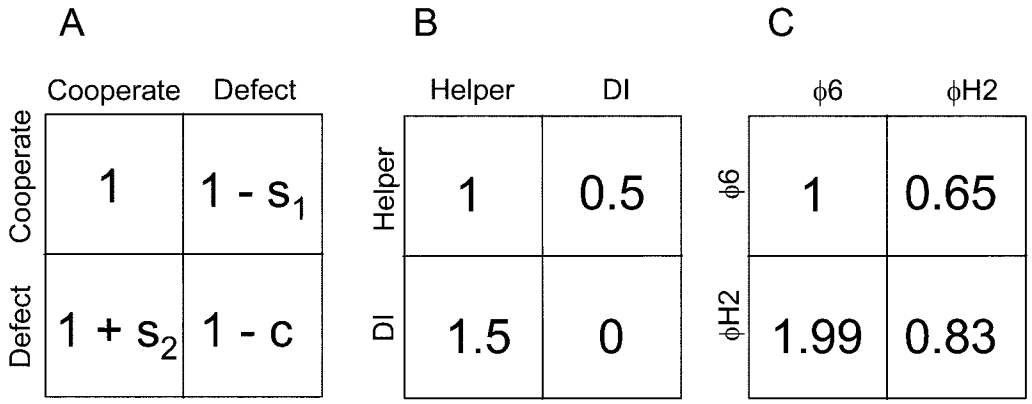

Figure 1: Payoff matrices for a game in which opponents utilize conflicting strategies of cooperation and defection. Entries in the payoff matrix represent the fitness to an individual on the left, when it encounters the above opponent. *A*, Generalized payoff matrix for a contest between cooperators and defectors. Defectors gain a fitness advantage $(1 + s_2)$ that allows them to invade a population of cooperators. If the cost of defection is too strong, $(1 - c) < (1 - s_1)$, cooperators may also invade, and the two strategies are driven to a stable polymorphism. Prisoner's Dilemma occurs if it always pays to be selfish: $(1 - c) > (1 - s_1)$; defection sweeps through the population despite the greater fitness payoff had all individuals cooperated. *B*, Hypothetical matrix for a contest between defective interfering (DI) particles and helper viruses. DI particles gain a replicative advantage over cooperative helper viruses, but DIs cannot exist on their own: $(1 - c) = 0$. Therefore, the only evolutionarily stable strategy for a DI is to coexist in a mixed polymorphism with a helper virus. *C*, Realized matrix for a contest between the ancestral cooperator (Φ6) and a virus evolved at high multiplicity for 200 generations (ΦH2). Because the selfish virus ΦH2 replaces the ancestor while simultaneously lowering the mean fitness of the population, these data are consistent with the Prisoner's Dilemma. Data in *C* are from Turner and Chao (1999).

specializes in appropriating a larger share of the products can be defined as a defector.

Defection in viruses is epitomized by the evolution of defective interfering (DI) particles, viruses that lack essential genes (or essential parts of the genome) and that interfere specifically with ordinary (helper) viruses by replicating at their expense (Huang and Baltimore 1970; Holland 1991; Vogt and Jackson 1999). In essence, DI particles are intracellular parasites that rely on functional proteins synthesized by coinfecting viruses. For this reason, DIs can only evolve when the environment contains high levels of coinfection, and their persistence is dependent on the continued presence of cooperators. To illustrate this outcome, consider a hypothetical payoff matrix for the interaction between a selfish DI particle and a helper virus (fig. 1*B*). Let $s_1 = s_2 = 0.5$ in this example. When the DI and the helper virus are paired, the helper is exploited and its fitness is $(1 - s_1) = 0.5$, whereas the DI benefits and its fitness is $(1 + s_2) = 1.5$. Therefore, the frequency of DIs is expected to increase in the population. However, by definition, a DI particle lacks a subset of the genes necessary for replication; therefore, c must equal 1.0 for any DI, and when these genotypes are paired their fitness is $(1 - c) = 0$. The latter would occur if DI particles were to outcompete (replace) helper viruses in the population. Thus, the only evolutionarily stable strategy available to a DI particle is a genetic polymorphism involving a helper. The appearance of DI particles is viewed as a nuisance in most virus experiments (Holland 1991); therefore, the long-term dynamics of DI–helper virus interactions are poorly studied (but see, e.g., Duhaut and Dimmock 1998). Full-length viruses may also cheat through mechanisms similar to those observed in DI particles, but examples are rare and mostly come from RNA viruses that infect plants (Vogt and Jackson 1999).

Bacteriophage (phage) Φ6 is a double-stranded RNA virus of the family Cystoviridae. Phage Φ6 infects the bacterium *Pseudomonas phaseolicola* and provides a useful model for the study of RNA virus evolution (Chao 1990; Turner and Chao 1998; Burch and Chao 1999) and molecular genetics (Mindich 1999). Although the evolution of DI particles is rare or nonexistent in Φ6, we recently observed that certain full-length genotypes of Φ6 behave selfishly. To examine competitive interactions among viruses, we conducted experiments that controlled the multiplicity of infection (MOI), or ratio of infecting phages to host cells (Turner and Chao 1998). A single clone of Φ6 was divided into three high-multiplicity (MOI = 5) and three low-multiplicity (MOI = 0.002) populations and allowed to evolve on *P. phaseolicola*. (Assuming Poisson sampling [Sokal and Rohlf 1981], at MOI = 5, coinfection by two or more viruses is common, and 97% of cells should experience multiple infections, whereas only 0.1% of all infected cells contain two or more viruses at MOI = 0.002.) High and low multiplicity in experimental populations was imposed for 50 consecutive days, or

roughly 250 generations of viral evolution. At the end of the experiment, population samples (stored in the freezer) were competed against a common competitor of the ancestral genotype to measure changes in fitness. Data showed that phage cultured at high multiplicity (but not low multiplicity) gain an added advantage during coinfection, suggesting that these viruses evolved a defection strategy for intracellular competition. However, in environments where high-multiplicity viruses become fixed and hence intracellular competition with other genotypes is removed, these phages exhibit evolution of lowered fitness. We later generated the payoff matrix for the high-multiplicity phage (defectors) relative to their ancestors (cooperators; fig. 1*C*; Turner and Chao 1999). These data provided evidence that Prisoner's Dilemma can evolve in a biological population: the selfish genotypes outcompeted their evolutionary progenitors while simultaneously lowering population fitness.

Our results indicate that when Φ6 is evolved under high multiplicities, the virus becomes trapped in a Prisoner's Dilemma (Turner and Chao 1999), suggesting that selfish genotypes may be readily isolated from populations of Φ6. However, very little is known regarding the natural biology of Φ6, including the opportunities for coinfection in the wild. Thus, it is important to examine the generality of this result and whether defectors are generally advantaged. The previous experiments allowed defectors to compete against their ancestors to prove that Prisoner's Dilemma can evolve in a single lineage. However, we define a cooperator as a virus that makes intracellular products in excess, and this productivity is expected to increase as viruses become better adapted to the host or culture conditions. Therefore, the solution to the payoff matrix in figure 1*C* may differ if evolved defectors are competed against evolved cooperators, viruses that are well adapted to *P. phaseolicola*. In theory, evolved cooperators may exist at a low frequency in one or more of the populations from which the defectors were originally isolated (Turner and Chao 1998). However, no genetic marker presently exists to easily distinguish defectors from cooperators, making it extremely difficult to retrieve evolved cooperators that may be present in these populations. The alternative is to obtain evolved cooperators by selecting for greater cooperation in the ancestral Φ6. One method is to propagate the ancestor under strictly clonal conditions (absence of competitive interactions), allowing viruses to become very efficient at making intracellular products. In reality, this selection experiment was previously completed; the low-multiplicity treatment in our original study allowed the ancestral cooperator to evolve for 250 generations in the absence of interactions with competing viruses (Turner and Chao 1998).

According to the model in figure 1*A*, the fitness of a defector is expected to be a decreasing function of its own initial frequency in competition. This is because defectors achieve their highest fitness when rare, but their fitness should decrease as the defectors more frequently encounter one another in the population. We will assume that the form of this fitness function is generally linear, as we observed previously (Turner and Chao 1999). We can identify three possible solutions to a game between evolved defectors and evolved cooperators (fig. 2). The first possibility is that fitness of the defector relative to the cooperator exceeds 1.0 at all initial frequencies of the defector. Thus, selfishness is the preferred strategy, and defectors will fix in the population, an outcome analogous to our previous report of Prisoner's Dilemma in evolving populations of Φ6 (fig. 1*C*; Turner and Chao 1999). The second possibility is the opposite extreme. If fitness of the defector relative to the cooperator is <1.0 at all initial frequencies, then defectors are never advantaged. Thus, cooperation is the preferred strategy. The third possibility is the more intriguing one. If the fitness function crosses 1.0, then defectors and cooperators are each advantaged when rare (fig. 2). This outcome indicates a mixed strategy. To examine the three contrasting outcomes, we present the results of experiments that compete the evolved defectors against their evolved cooperative counterparts.

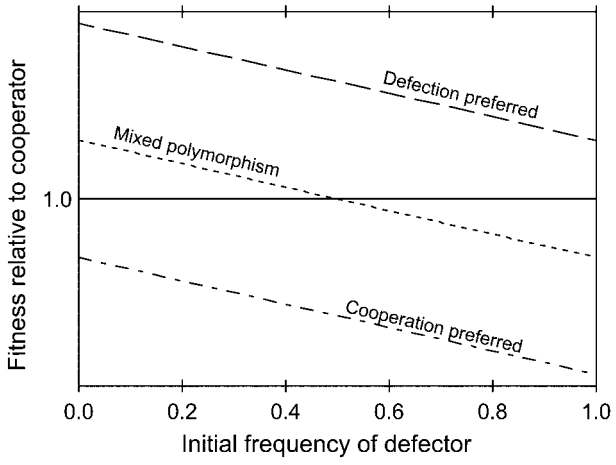

Figure 2: Hypothetical outcomes when defector and cooperator viruses are competed at different initial frequencies in the presence of coinfection. The model assumes that the dependence of relative fitness on initial frequency is a linear function. If defectors are advantaged at all initial frequencies, then defection is the preferred strategy (*dashed line*). If cooperators are always advantaged, then cooperation is preferred (*dashed and dotted line*). If neither strategy is able to resist invaders, then a mixed polymorphism results (*dotted line*).

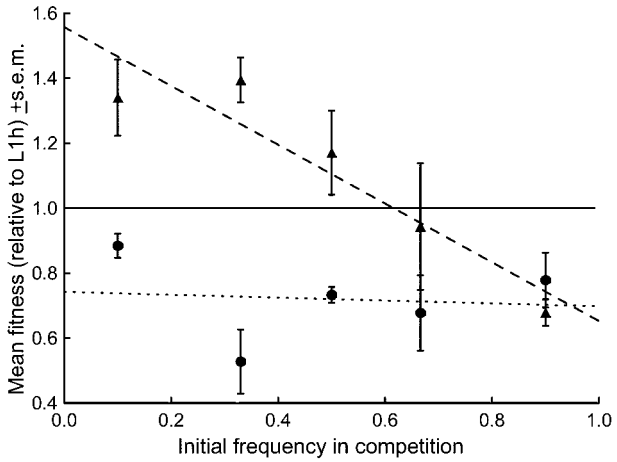

Figure 3: The fitness of derived defectors relative to evolved cooperators is frequency dependent only when intrahost competition is allowed. Phages ΦH2 and ΦL1 were competed at five initial frequencies of ΦH2 at multiplicity of infection (MOI) = 5 (*triangles*) and at MOI = 0.002 (*circles*). Each point is the mean ± SEM of three replicate competitions. Linear regression analysis shows that the fitness of ΦH2 is a decreasing function of its own initial frequency at high MOI (*dashed line*) but that ΦH2 is generally disadvantaged at all initial frequencies at low MOI (*dotted line*). See "Results" for statistics.

Material and Methods

Strains

From an experiment previously described (Turner and Chao 1998), the viral clones ΦH2 and ΦL1 were isolated at generation 200 from a population evolved at MOI = 5 and MOI = 0.002, respectively. We obtained a spontaneous host-range mutant of ΦL1, referred to as ΦL1h. Host-range ability occurs through a point mutation on the medium segment, and preliminary experiments showed that the h marker imposed a 6% fitness cost under our experimental conditions (data not shown). All fitness measurements relative to ΦL1h reported below are adjusted to reflect the cost of the h marker.

The typical laboratory host of Φ6 is *Pseudomonas phaseolicola* (Vidaver et al. 1973), obtained from the American Type Culture Collection (ATCC 21781). L. Mindich (Public Health Research Institute, New York) kindly provided the alternative host *Pseudomonas pseudocaligenes* (East River, New York, isolate A; Mindich et al. 1976*b*). Phage ΦL1h forms clear plaques when plated on a mixed lawn containing both *P. phaseolicola* and *P. pseudocaligenes*, whereas non-host-range phages form turbid plaques on a mixed lawn because they do not kill the *P. pseudocaligenes* cells.

Media and Culture Conditions

All phages and bacteria were grown, plated, incubated, and diluted at 25°C in LC medium, a modification of Luria broth (Sinclair et al. 1976). Bacterial cultures were inoculated by a single bacterial colony placed into 10 mL LC medium in a sterile flask. Culture flasks were grown for 24 h in a shaking incubator at 25°C and 120 rpm, during which bacterial cultures attained stationary-phase densities (~4×10^9 cells/mL for *P. phaseolicola* and ~5×10^{10} cells/mL for *P. pseudocaligenes* ERA).

Agar concentrations in plates were 1.5% and 0.7% for bottom and top LC agar, respectively. Volume of top agar was 3 mL/plate, and that of bacterial lawns was 200 μL. Lawns were made from overnight bacterial cultures of *P. phaseolicola* or a mixture of *P. phaseolicola* and *P. pseudocaligenes* ERA at a 200 : 1 volumetric ratio where ordinary and host-range phages produce turbid and clear plaques, respectively.

Plaque lysates were prepared by plating plaque-purified phage with top agar and a *P. phaseolicola* lawn. After 24 h, plaques in the top agar were resuspended in 3 mL of LC broth and centrifuged at 3,000 rpm for 10 min. Supernatant containing the phage lysate was filtered (0.22 μm, Durapore, Millipore) to remove bacteria. Phage lysates and bacteria stocks were stored in 4 : 6 glycerol/LC (v/v) at −20°C.

Fitness Assays

Ordinary and host-range phages were mixed at a defined ratio and allowed 40 min adsorption to *P. phaseolicola* at MOI = 5, or MOI = 0.002. From the adsorption mixture, roughly 500 infected cells were plated with top agar and a *P. phaseolicola* lawn. After 24 h, plaques were visible, and a filtered lysate was prepared as above. We monitored the ratio of phages in the starting mixture (R_0) and that in the harvested lysate (R_1) by plating on a mixed lawn containing *P. phaseolicola* and *P. pseudocaligenes* (200 : 1 ratio), where the ratio of the two phages was based on the h marker. Fitness (W) was defined as $W = R_1/R_0$.

Results

Fitness of Defectors is Frequency Dependent Only at High MOI

Game theory predicts that defectors achieve their highest fitness when they are rare and interacting primarily with cooperators (fig. 1*A*). To confirm that fitness of evolved defectors relative to evolved cooperators was dependent on their initial frequency in competition, we competed ΦH2 against ΦL1 at five initial frequencies of ΦH2 (0.1, 0.33, 0.5, 0.66, 0.9) at MOI = 5, with replication (n =

3). Results (fig. 3) clearly show that the fitness of ΦH2 is a decreasing function of its own initial frequency at MOI = 5. A linear model provided a statistically significant fit to the data (linear regression with $F = 21.367$, df = 1, 3, $P = .019$, $r^2 = 0.8769$). To determine whether a higher-order (curvilinear) model better describes the data, we tested whether the incorporation of a quadratic term significantly improved the model's fit (Kleinbaum and Kupper 1978). This analysis showed that a curvilinear relationship was not statistically supported ($F = 1.9887$, df = 2, 2, $P > .25$). We concluded that a linear function best described the frequency-dependent fitness of ΦH2 relative to ΦL1, a result similar to that observed when ΦH2 was competed against its ancestor Φ6 (Turner and Chao 1999).

In contrast, an identical experiment where the two genotypes were competed at MOI = 0.002 (fig. 3) shows no evidence for frequency dependence (linear regression with $F = 0.0323$, df = 1, 3, $P = .869$, $r^2 = 0.0107$). Rather, ΦH2 is generally disadvantaged at all initial frequencies; fitness of ΦH2 at MOI = 0.002 does not differ according to initial frequency (one-way ANOVA with MSE = 0.059, df = 4, $F_S = 2.678$, $P = .094$), yielding a mean fitness relative to ΦL1 of $W = 0.720 \pm 0.059$ SEM. We concluded that fitness of ΦH2 was frequency dependent only at MOI = 5, an environment where the virus gains an advantage during intrahost competition.

Demonstration of Mixed Polymorphism

The data in figure 3 demonstrate that evolved cooperators and evolved defectors will coexist in a mixed polymorphism at MOI = 5. By definition, coexistence will occur when the two genotypes achieve frequencies in the population where they are equally fit. This predicted frequency for the evolved defector is easily obtained by noting the point where the regression line intersects a relative fitness of 1.0 and extrapolating to the corresponding value on the *X*-axis. Hence, the predicted frequency of ΦH2 is approximately 0.62 in the polymorphism.

Furthermore, these data can be used to illustrate a mixed polymorphism described by solving the payoff matrix in figure 1*A*. Fitness was measured at a high MOI where most phages reproduce in coinfected cells. At low initial frequencies of ΦH2, pure coinfections containing only ΦH2 are rare, and the fitness of ΦH2 is primarily determined by mixed coinfections that also contain ΦL1. Thus, the fitness of ΦH2 at low frequencies is equal to $(1 + s_2)$. It follows that ΦL1 is very abundant at low frequencies of ΦH2, and most coinfection events will occur through the cooperator alone. If the fitness of ΦL1 in a pure coinfection is defined to be 1, then the *Y*-intercept of the regression line at MOI = 5 equals $(1 + s_2)/1$, which provides the estimate of $(1 + s_2) = 1.55$. By the same logic, the fitnesses of ΦH2 and ΦL1 at high initial frequencies of ΦH2 are $(1 - c)$ and $(1 - s_1)$, whereby the ratio $(1 - c)/(1 - s_1)$ is estimated by the extrapolated fitness value in figure 3 at a frequency of 1.0. Because the extrapolated value of 0.66 is <1.0, $(1 - c) < (1 - s_1)$ as required by a mixed polymorphism.

To complete the payoff matrix, we performed an additional competition experiment to measure $(1 - c)$. Adsorption is the step in phage infection that involves attachment and entry of viruses into a bacterial cell (Stent 1963). We modified the fitness assay such that each phage was allowed to adsorb separately at MOI = 5, and then we mixed the adsorbed phage (each at a frequency of 0.5) just before plating the fitness assay. This manipulation differs from the previous experiments (fig. 3) in which the phages were mixed before adsorption. Therefore, whereas previous assays involved cells coinfected by both ΦH2 and ΦL1, this split assay contains only cells infected with either ΦH2 or ΦL1 genotypes. In the absence of mixed infection, the fitness of ΦH2 is $(1 - c)$ and that of ΦL1 is 1 (fig. 1*A*). Results showed $(1 - c)$ to be 0.374 ± 0.206 SEM ($n = 5$), whereby $(1 - s_1) = 0.37/0.66 = 0.56$. We note that in the above assays involving mixed infection, the fitness of ΦH2 is much higher at a frequency of 0.5 (cf. fig. 3). Figure 4*A* shows the completed payoff matrix in a competition between ΦH2 and ΦL1 at MOI = 5.

It is instructive to adjust the payoff matrix in figure 4*A* to reflect fitness of the evolved cooperator and defector relative to their common ancestor Φ6. In split assays, we previously showed that the fitness of the evolved defector ΦH2 relative to the ancestral cooperator Φ6 is $(1 - c) = 0.83$ (fig. 1*C*; Turner and Chao 1999), where the fitness of the ancestor is 1, by definition. The resulting equation is $W_{\text{ancestor}}/W_{\text{defector}} = 1/0.83$; therefore, $W_{\text{defector}} = (W_{\text{ancestor}} \times 0.83)$. Similarly, in the current experiment $W_{\text{cooperator}}/W_{\text{defector}} = 1/0.37$. What then is the adjusted fitness of the evolved cooperator when paired with itself in the context of the new payoff matrix in figure 4*A*? This is obtained by substitution: $W_{\text{cooperator}} = (W_{\text{ancestor}} \times 0.83)/0.37$, where $W_{\text{cooperator}} = 2.24$. Thus, we can scale the new matrix by multiplying each fitness value by 2.24.

The scaled matrix in figure 4*B* shows that the cost of defection, $(1 - c) = 0.83$, has not changed from that observed in our earlier experiments (fig. 1*C*; Turner and Chao 1999). In fact, this value could not have changed because the defectors were not allowed to evolve further. But it is revealed that defectors now enjoy an even greater advantage when interacting with cooperators: $(1 + s_2) = 3.47$. Our results also show that the cost of being a cooperator has decreased: $(1 - s_1) = 1.25$. However, because $(1 - c) < (1 - s_1)$, the scaled payoff matrix still predicts that

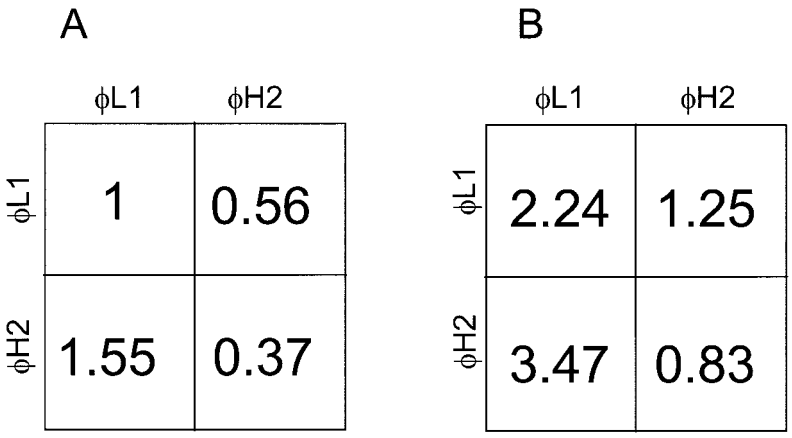

Figure 4: *A*, Realized payoff matrix for a contest between the evolved cooperator (ΦL1) and the evolved defector (ΦH2) reveals a mixed strategy. Observed fitness values indicate that the selfish virus ΦH2 has a large fitness advantage when it is initially rare, and it should increase in frequency in a population of evolved cooperators. However, the lowest payoff is to an evolved defector during coinfection with a similar genotype. Thus, an evolved cooperator is also advantaged when rare, producing a mixed polymorphism involving the two strategies. *B*, Adjusted payoff matrix scaled relative to the common ancestor of both evolved genotypes. Although the cost of defection (c) is unchanged, the benefit of defection (s_2) has increased, and the cost of cooperation (s_1) has decreased (cf. fig. 1*C*).

cooperators and defectors will coexist in a mixed polymorphism.

Discussion

Evolution of phage Φ6 at high multiplicities selects for genotypes that gain a fitness benefit during coinfection (Turner and Chao 1998), but fixation of these viruses causes mean fitness of the population to decline. These combined results are consistent with the evolution of lowered fitness in a population of defectors as expected from the Prisoner's Dilemma (Turner and Chao 1999). Is the Prisoner's Dilemma a given in Φ6 (i.e., a constraint of the system)? Or will Prisoner's Dilemma break down as virus genotypes are evolved at other multiplicities?

We allowed the ancestral Φ6 cooperator to be grown under strictly clonal conditions (absence of competitive interactions), creating the opportunity for greater cooperation to evolve. When these evolved cooperators were competed against the evolved defectors, our results showed that the two strategies should coexist in a mixed polymorphism. Thus, our data suggest that phage Φ6 is unlikely to be permanently trapped in a Prisoner's Dilemma. Rather, evolution of Prisoner's Dilemma is likely to be a local phenomenon for populations experiencing high rates of coinfection. If evolved cooperators were to enter these populations, our data show the locally adapted defectors would face a mixed polymorphism at best. That is, our fitness data at low multiplicities indicate that if the local environment changes such that only clonal infections occur, then the preferred strategy would switch to 100% cooperation and the defectors would disappear. Rates of coinfection for Φ6 in the wild are unknown. However, Φ6 features an exclusion mechanism where only three viruses on average are able to infect the cell, even though up to 50 viruses can attach to the cell (Olkkonen and Bamford 1989; Turner et al. 1999). This argues that exclusion may be a Φ6 adaptation to reduce the level of intrahost competition, suggesting that coinfection occurs readily enough to be a target of selection.

The payoff matrix accompanying a Prisoner's Dilemma features $(1 - c) > (1 - s_1)$, where defection sweeps through the population. In contrast, a mixed polymorphism features $(1 - c) < (1 - s_1)$, where cheaters are prevented from taking over. In general, the transition from a Prisoner's Dilemma to a mixed polymorphism can be achieved either by selection for more cheating and the associated costs (i.e., increasing c) or by selection for decreased sensitivity to cheaters (i.e., decreasing s_1). Our experiments indicate that the latter is a possibility in Φ6. Thus, the mixed polymorphism was achieved because levels of cooperation are variable in this system. The payoff matrix of game theory describes pairwise interactions between individuals utilizing discrete behaviors, and we treat the strategies of cooperation and defection in Φ6 as essentially static (Turner and Chao 1999). This approach is justified because an individual phage genotype is unable to modify its behavior or to switch between strategies. However, we allowed one player (the cooperator) to evolve, and accordingly, its modified behavior led to a change in the matrix outcome.

Our results show that evolution of one player can change the quantitative values of the matrix to yield a qualitatively different outcome. In our previous study demonstrating the Prisoner's Dilemma (Turner and Chao 1999), only a single strategy (defection) was preferred. In the current matrix, both defection and cooperation are predicted to be evolutionarily stable. This change in the payoff matrix contrasts with a model and analysis by Brown (2001), in which constraints on the cost and payoff of cooperation and defection lead to evolution of only a single preferred strategy (defection).

Phage Φ6 has a double-stranded RNA genome that is split into three smaller segments (denoted small, medium, and large; Semancik et al. 1973). Defective interfering (DI) particles typically feature one or more gene deletions that allow these genotypes to gain a replicative advantage over ordinary full-length viruses (Holland 1991). Although selfish DI particles are unknown in Φ6, our data suggest that selfishness can evolve in full-length forms of the virus. It can be argued that the temptation to cheat is a nearly universal phenomenon in biology (Axelrod 1985; Dugatkin 1997). Our data support this idea. Barriers to DI evolution may exist in Φ6 (although this is uncertain), but we observed that selfishness readily evolves in full-length viruses propagated at high multiplicity (Turner and Chao 1998).

Potential Mechanisms for Selfishness

The mechanism that affords high-multiplicity viruses an advantage during coinfection remains unknown, but studies on other viruses suggest several possibilities. One is preferential encapsidation. The Φ6 genome is divided into three RNA segments (Semancik et al. 1973). During intracellular reproduction, empty viral capsids are generated and replicated segments enter the capsid in an ordered fashion (Qiao et al. 1997). Genetic exchange (sex) can occur when multiple viruses coinfect the same host cell and hybrid progeny are generated through random reassortment of segments found in the coinfecting parents (Mindich et al. 1976*a*). Because recombination among individual segments is very rare or unknown in Φ6 (Mindich et al. 1976*a*), the genes on each segment are effectively linked. Selfishness can evolve if a selfish gene biases the entry of its resident segment into available capsids, a process analogous to meiotic drive in eukaryotes. That is, a selfish virus would reduce the probability that other coinfecting genotypes contribute the expected share of their genes to the progeny. This hypothesis could be tested by comparing the observed frequency of hybrids generated in crosses involving selfish viruses to that expected through the Poisson (Turner et al. 1999). Certain DI particles of RNA viruses can gain a replicative advantage through gene duplications involved in encapsidation (Holland 1991). Extra sequences recognized by encapsidation enzymes could explain why selfish viruses replicate slowly at low multiplicities but gain a fitness advantage via increased encapsidation at high multiplicities.

A second possible mechanism is a trade-off between replication and transcription in selfish viruses (Holland 1991; Chao et al. 2000). Because a viral genome serves as a template for both 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 is common, one virus can evolve to allocate more to replication and come to rely on other viruses to produce the required proteins. Viruses that specialize in protein production are effectively cooperators, and ones that specialize in replication are defectors. One possibility for the polymorphism is therefore that the low-multiplicity viruses evolved to synthesize larger quantities of intracellular products than the high-multiplicity viruses. Such larger amounts could then have decreased the value of s_1, or the sensitivity of the low-multiplicity viruses to parasitism from the high-multiplicity viruses. Whereas the ancestral population succumbs to invasion by defecting mutants, derived cooperators that generate an increased resource pool could tolerate a subpopulation of selfish individuals that synthesize less but specialize in sequestering a larger share of the products.

Alternative Explanations

Cooperation and defection offer a highly plausible explanation for our results, given the precedence in RNA viruses (von Magnus 1954; Huang and Baltimore 1970; Holland 1991; Bonhoeffer and Nowak 1994). But competitive exclusion provides an alternative explanation. Competitive exclusion results from either exploitation or interference. Exploitation occurs when one genotype uses the limiting resource more efficiently than other genotypes, which are suppressed indirectly through the depletion of the resource. Interference occurs when one genotype directly inhibits the survival or reproduction of other genotypes, for example, by producing allelopathic (anticompetitor) toxins. Because the cost of exploitation competition is built into the advantage, it cannot be disassociated from the benefits. Thus, competitive exclusion by exploitation does not predict our observed relationship between fitness and the frequency of the evolved virus (fig. 3; see also Turner and Chao 1999). However, interference competition can incur a cost that can be separated from the benefits. Producing allelopathic toxins incurs a cost but yields no benefits when toxin-sensitive competitors are absent. Thus, competitive exclusion by interference can also explain our frequency dependence.

There are many examples of interference competition in microbes and viruses. In a previous article, we (Chao and Levin 1981) have documented the frequency-dependent selection for bacteria producing allelopathic compounds known as colicins. In the latter case, it is competitive exclusion by interference that generates the frequency dependence. It also seems reasonable that viruses encoding for a restriction endonuclease would gain an advantage analogous to producing an allelopathic compound. If bearing restriction endonucleases incurs a cost, selection should also be frequency dependent. Because we have not worked out the basis of the frequency-dependent selection for our evolved bacteriophage, we cannot reject the relevance of competitive exclusion by interference (or other possible mechanisms). However, previous work with RNA viruses has clearly demonstrated the existence of a variety of mechanisms equivalent to cooperation and defection, and we currently favor a qualitatively similar explanation.

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Literature Cited

Axelrod, R. 1985. The evolution of cooperation. Basic, Boulder, Colo.

Bonhoeffer, S., and M. A. Nowak. 1994. Intra-host versus inter-host selection: viral strategies of immune function impairment. Proceedings of the National Academy of Sciences of the USA 91:8062–8066.

Brown, S. P. 2001. Collective action in an RNA virus. Journal of Evolutionary Biology 14:821–828.

Burch, C. L, and L. Chao. 1999. Evolution by small steps and rugged landscapes in the RNA virus phi-6. Genetics 151:921–927.

Chao, L. 1990. Fitness of RNA virus decreased by Muller's ratchet. Nature 348:454–455.

Chao, L., and B. R. Levin. 1981. Structured habitats and the evolution of anticompetitor toxins in bacteria. Proceedings of the National Academy of Sciences of the USA 78:6324–6328.

Chao, L., K. A. Hanley, C. L. Burch, C. Dahlberg, and P. E. Turner. 2000. Kin selection and parasite evolution: higher and lower virulence with hard and soft selection. Quarterly Review of Biology 75:261–275.

Dugatkin, L. A. 1997. Cooperation among animals: an evolutionary perspective. Oxford University Press, Oxford.

Duhaut, S. D., and N. J. Dimmock. 1998. Heterologous protection of mice from a lethal human H1N1 influenza A virus infection by H3N8 equine defective interfering virus: comparison of defective RNA sequences isolated from the DI inoculum and mouse lung. Virology 248: 241–253.

Holland, J. 1991. Defective viral genomes. Pages 151–165 *in* B. Fields and D. Knipe, eds. Fundamental virology. 2d ed. Raven, New York.

Huang, A. S., and D. Baltimore. 1970. Defective viral particles and viral disease processes. Nature 226:325–327.

Kleinbaum, D. G., and L. L. Kupper. 1978. Applied regression analysis and other multivariable methods. Duxbury, North Scituate, Mass.

Maynard Smith, J. 1982. Evolution and the theory of games. Cambridge University Press, Cambridge.

Mindich, L. 1999. Reverse genetics of dsRNA bacteriophage phi-6. Advances in Virus Research 53:341–353.

Mindich, L., J. F. Sinclair, D. Levine, and J. Cohen. 1976*a*. Genetic studies of temperature-sensitive and nonsense mutants of bacteriophage Φ6. Virology 75:218–223.

Mindich, L., J. Cohen, and M. Weisburd. 1976*b*. Isolation of nonsense suppressor mutants in *Pseudomonas*. Journal of Bacteriology 126:177–182.

Nee, S. 2000. Mutualism, parasitism, and competition in the evolution of coviruses. Philosophical Transactions of the Royal Society of London B, Biological Sciences 355:1607–1613.

Nee, S., and J. Maynard Smith. 1990. The evolutionary biology of molecular parasites. Parasitology 100(suppl.): S5–S18.

Olkkonen, V. M., and D. H. Bamford. 1989. Quantitation of the adsorption and penetration stages of bacteriophage Φ6 infection. Virology 171:229–238.

Qiao, X., J. Qiao, and L. Mindich. 1997. Stoichiometric packaging of the three genomic segments of dsRNA bacteriophage Φ6. Proceedings of the National Academy of Sciences of the USA 94:4074–4079.

Semancik, J. S., A. K. Vidaver, and J. L. Van Etten. 1973. Characterization of a segmented double-helical RNA from bacteriophage Φ6. Journal of Molecular Biology 78:617–625.

Sinclair, J. F., J. Cohen, and L. Mindich. 1976. The isolation of suppressible nonsense mutants of bacteriophage Φ6. Virology 75:198–208.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. 2d ed. W. H. Freeman, San Francisco.

Stent, G. 1963. Molecular biology of bacterial viruses. W. H. Freeman, San Francisco.

Turner, P. E., and L. Chao. 1998. Sex and the evolution of intrahost competition in RNA virus Φ6. Genetics 150: 523–532.

———. 1999. Prisoner's dilemma in an RNA virus. Nature 398:441–443.

Turner, P. E., C. Burch, K. Hanley, and L. Chao. 1999. Hybrid frequencies confirm limit to coinfection in the RNA bacteriophage Φ6. Journal of Virology 73: 2420–2424.

Vidaver, K. A., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage Φ6: a lipid-containing virus of *Pseudomonas phaseolicola*. Journal of Virology 11:799–805.

Vogt, P. K., and A. O. Jackson, eds. 1999. Satellites and defective viral RNAs. Current Topics in Microbiology and Immunology 239. Springer, New York.

von Magnus, P. 1954. Incomplete forms of influenza virus. Advances in Virus Research 2:59–79.

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