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Competitive fates of bacterial social parasites: persistence and self-induced extinction of *Myxococcus xanthus* cheaters

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Cooperative biological systems are susceptible to disruption by cheating. Using the social bacterium *Myxococcus xanthus*, we have tested the short-term competitive fates of mixed cheater and wild-type strains over multiple cycles of cooperative development. Cheater/wild-type mixes underwent several cycles of starvation-induced multicellular development followed by spore germination and vegetative population growth. The population sizes of cheater and wild-type strains in each pairwise mixture were measured at the end of each developmental phase and each growth phase. Cheater genotypes showed several distinct competitive fates, including cheater persistence at high frequencies with little effect on total population dynamics, cheater persistence after major disruption of total population dynamics, self-extinction of cheaters with wild-type survival, and total population extinction. Our results empirically demonstrate that social exploitation can destabilize a cooperative biological system and increase the risk of local extinction events.

Keywords: cheating; chicken game; cooperation; extinction; levels of selection; myxobacteria

1. INTRODUCTION

Many higher organisms contribute to cooperative groups at some individual cost, in turn receiving a benefit from the group that they could not procure as isolated individuals (Alexander 1974; Wilson 1975; Seeley & Visscher 1988; Krebs & Davies 1997; Sober & Wilson 1998; Watts & Mitani 2001; Clutton-Brock 2002; Wedekind & Braithwaite 2002). Such cooperative systems are susceptible to exploitation by individuals that partially or completely withhold their costly individual contributions, while simultaneously receiving a disproportionately high cooperation-based benefit (Axelrod & Hamilton 1981; Hölldobler & Wilson 1990; Maynard Smith & Szathmáry 1995; Keller 1999; Abbot *et al.* 2001). The competitive fates of exploited social groups and their cooperative and selfish subpopulations are determined by multiple parameters, including the degree to which cooperation is a major fitness component, the intensity of exploitation, and the extent of cooperation among exploiters when they are common (Sober & Wilson 1998; Michod 1999). If social exploitation results in major population-size fluctuations, it may increase the likelihood of local population extinctions.

Social exploitation strategies can be evolutionarily facultative or obligate (MacDonald & Matthews 1975; Fisher 1987; Bourke & Franks 1995; Velicer 2003). Facultatively selfish individuals are proficient among themselves at the very social behaviour that they exploit in their victims. For example, individuals of the social aphid *Pemphigus obesinymphae* facultatively adopt selfish behavioural strategies after infiltrating a colony of non-kin, but remain altruistic when surrounded by close kin in their colony of origin (Abbot *et al.* 2001). In the presence of exploitable victims, facultative exploiters can be expected to increase their relative frequency while having a relatively small effect on the total size of populations containing both exploiters and their victims.

Alternatively, evolutionarily obligate cheaters require the presence of their social host for evolutionary success. On their own, they are defective at the social behaviour that they exploit in other genotypes. For example, queens of some *Polistes* wasp species and of many ant species produce only sexual offspring and parasitically depend on previously established workforces of closely related genotypes to raise their brood (Hölldobler & Wilson 1990; Turillazzi *et al.* 1990; Fanelli *et al.* 2001). When present at high frequencies, obligate cheaters are predicted to cause major collapses of combined cheater–altruist populations owing to their poor performance at an exploited social trait that is important for reproductive success (Sturtevant 1938).

Some micro-organisms have been shown to exhibit cooperative, altruistic and exploitative behaviours that are analogous to similar behaviours in higher eukaryotes (Chao & Levin 1981; Zahavi & Ralt 1984; Turner & Chao 1999; Strassmann *et al.* 2000; Velicer *et al.* 2000; Crespi 2001; Vulic & Kolter 2001; Velicer 2003). Here, we used the social bacterium *Myxococcus xanthus* to examine the population-level effects and competitive fates of obligate social cheaters in extended competitions between cheater and altruistic genotypes during sequential exposures to conditions where social cooperation is important for survival.

The myxobacteria are a monophyletic group of social bacteria characterized by their ability to develop cooperatively into complex fruiting structures during nutrient deprivation (Shimkets & Woese 1992). Under some conditions, this process of multicellular development is necessary for mere survival. Most myxobacteria are soil-dwelling predators that swarm as groups toward prey

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organisms, killing and decomposing them with a diverse arsenal of secretions (Rosenberg & Varon 1984). Upon depletion of amino acids in the local environment, swarms of *M. xanthus* aggregate into groups of *ca*. 10^5 organisms and embark on a communicative process of fruiting body formation. Functional motility and several stage-specific intercellular signal molecules are required to complete the developmental process (Shimkets 1999). Within the fruiting body, a minority of the population transforms from vegetative rods into tough, spherical spores. The remaining cells either undergo autolysis or become peripheral rods that surround the fruiting body perimeter (Wireman & Dworkin 1977; O'Connor & Zusman 1991*a*,*b*). It has been hypothesized that autolysis provides resources that benefit the sporulating minority, but a clearly adaptive role for autolysis has yet to be demonstrated (Shimkets 1999).

Myxococcus xanthus fruiting body development and sporulation result largely from the collective action of individuals within aggregate groups that generate a common pool of intercellular signals (Shimkets 1999). Such fruiting body development is conceptually similar to group pathogenic infections in which 'the exploitative rate of an individual parasite…is limited by the collective action of the coinfecting group' (Brown *et al.* 2002, p. 402). Such group limitation of fitness is a result of group members sharing a pool of fitness-limiting resources (e.g. RNA replicase in viral infections or developmental signals in *M. xanthus*) that are generated by group members themselves. In recent studies, investigators have modelled the effects of relatedness within coinfecting groups on virulence when the reproductive rate of individuals is limited by the collective action of all group members (Chao *et al.* 2000; Brown *et al.* 2002; West & Buckling 2003). Because genetic heterogeneity increases the likelihood of interference competition and social cheating, low within-group relatedness can decrease the collective effect (virulence in this case) of an infecting group. Alternatively, high relatedness within groups where individual fitness is limited by collective behaviour (e.g. clonal fruiting bodies of *M. xanthus*) should result in relatively high cooperative output (spore production in this case). Within-group heterogeneity, in contrast, encourages selfish behavioural strategies that can lead to suboptimal population dynamics (e.g. low spore production) owing to mitigated collective action.

In two previous studies, six *M. xanthus* strains that cheat on their parental wild-type strain in mixed populations undergoing development were identified (Velicer *et al.* 2000, 2002). These cheater genotypes are each defective, to varying degrees, at spore production in pure culture, and yet produce a disproportionately high number of spores relative to their initial frequency in mixtures with the altruistic wild-type strain. (In the mixed competitions described here, the wild-type strains are altruistic because they produce, at their own expense, developmental signals that are beneficial to cheaters.) The cheaters include genotypes that evolved under asocial laboratory conditions (Velicer *et al.* 1998) as well as experimentally generated defined mutants that fail to produce an essential developmental signal. In this study, we examine the competitive fates of three of these cheaters over sequential cycles of development. In pairwise mixtures of wild-type and cheater genotypes, we quantified changes in the frequencies of both subpopulations over several cycles of development and vegetative growth, as well as fluctuations in total population size.

The three cheater genotypes examined here were chosen because they are severely defective at sporulation in pure culture (Velicer *et al.* 2000, 2002). Thus, they are obligate social parasites that depend on the presence of wild-type altruists for evolutionary success under conditions of strong selection for developmental proficiency. All three cheaters cause dramatic decreases in total-population spore production (during a single developmental cycle) when mixed at high frequencies with the wild-type strain by direct manipulation (Velicer *et al.* 2000, 2002; this study). Here, we test whether these cheaters also rise of their own accord to disruptively high frequencies during multi-cycle competitions, after having begun the competitions as a small minority (1%). We also examine what effect such disruptions may have on the competitive fates of both the cheaters and their altruistic competitors.

The social interactions of microorganisms can be analysed using evolutionary game theory (Axelrod & Hamilton 1981; Turner & Chao 1999; Lenski & Velicer 2000; Vulic & Kolter 2001; Velicer 2003). Such analysis of frequency-dependent cheater fitness in *M. xanthus* predicts that, in the absence of evolutionary change or cheater-induced extinction events, all three cheaters and the wild-type variants should persist as balanced polymorphisms over repeated cycles of development. All three cheaters conform to the so-called 'chicken game' matrix of frequency-dependent fitness relationships to the wild-type. In the chicken game, defection from cooperation (the cheaters' strategy) is advantageous when defectors are rare, but cooperation (the wild-type strategy) confers higher fitness when defectors are common (Bornstein *et al.* 1997). Our cheaters sporulate more efficiently than the wild-type variants when rare, but less efficiently when common (Velicer *et al.* 2000, 2002).

2. MATERIAL AND METHODS

(a) *Strains*

Three developmentally proficient, altruistic wild-type (W) variants and three developmentally defective cheater (C) clones of *M. xanthus* were used in this study. Strains W1, W2 and W3 are variants of the commonly studied strain DK1622 (Kaiser 1979). W1 is sensitive to all antibiotics used in this study, W2 differs from its parent W1 in terms of a spontaneous mutation conferring resistance to rifampicin, and W3 differs from W1 in terms of the presence of a chromosomally integrated plasmid (pDW79; Wall *et al.* 1999) that confers kanamycin resistance. (Strains W1 and W2 correspond to strains S and R, respectively, in Velicer *et al.* 1998 and 2002.) Three previously described cheater genotypes, LS523 (Shimkets & Asher 1988), GVB206.3 (Velicer *et al.* 2000) and S2/pDW79/- (Velicer *et al.* 2002) are denoted here as C1, C2 and C3, respectively, for simplicity. Strain C1 is a defined mutant of DK1622 that fails to produce C-signal (a primary developmental signal in *M. xanthus*) and is resistant to oxytetracycline. Strain C2 is a rifampicin-resistant evolutionary derivative of W2, and C3 is an evolutionary descendant of W1 marked with kanamycin resistance by integration of the plasmid pDW79. The developmental defects of strains C2 and C3 have not been characterized at the molecular

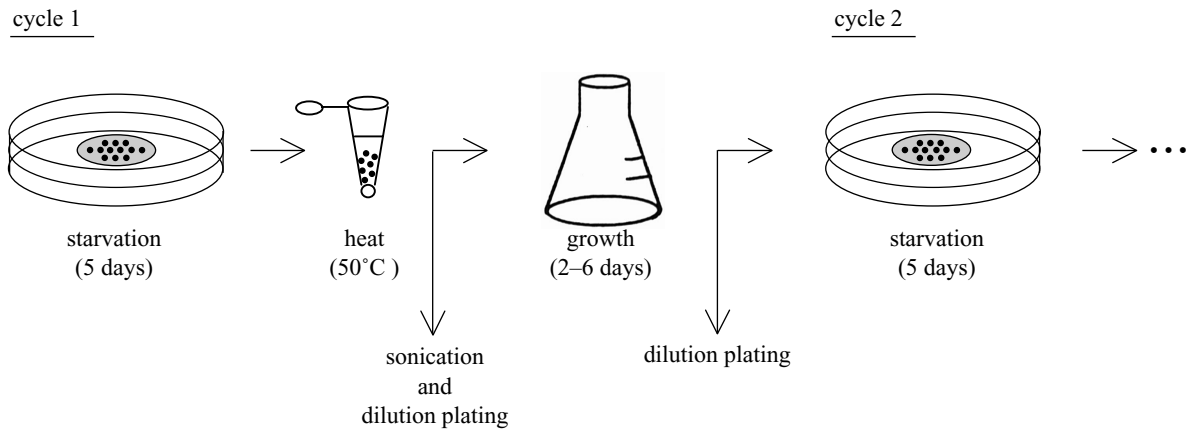

Figure 1. Competition cycle diagram. See text for description. Filled circles represent fruiting bodies.

level. These strains are also defective in group motility and exhibit faster growth in liquid culture than their evolutionary ancestors (Velicer *et al.* 1998, 2002).

(b) *Competition assays*

(i) *Competition initiation*

Competing strains were inoculated into CTT liquid (Kaiser 1979), grown to high density, diluted and grown in pure culture overnight, centrifuged (15 min at 4500*g*) at room temperature and the pellets resuspended to 5×10^9 cells ml^{-1} in TPM buffer (Kroos *et al.* 1986). In all competitions, strain pairs (W1/W2, W1/W3, W2/C3, W3/C1, C1/W3 and W3/C2) were mixed from resuspended cultures at a 99 : 1 ratio with the first-listed strain in the majority. Aliquots of 100 µl of the mixed resuspensions (5×10^8 cells total) were spotted at the centre of TPM agar plates (1.5% agar) for starvation and allowed to dry.

(ii) *Transfer cycles*

The TPM starvation plates were incubated for 5 days (32 °C, 90% relative humidity), at which time the populations were harvested with a scalpel blade, transferred into 1 ml twice-distilled H_2O, and heated at 50 °C for 2 h to select for viable spores (figure 1). An aliquot (500 µl) of each heated sample was transferred into 5 ml CTT liquid. All liquid cultures were grown at 300 rpm; 32 °C. Spore populations in liquid culture germinated and vegetative cells were grown to high density (greater than 2×10^8 cells ml^{-1}) in the growth phase before being centrifuged as above, resuspended in TPM liquid to 5×10^9 cells ml^{-1}, and spotted in 100 µl aliquots onto fresh TPM agar plates. Thus, all developmental populations in all experiments began development at equal population size (5×10^8 cells) and density. Liquid cultures inoculated with samples bearing large spore populations (i.e. near wild-type levels) grew to high density within 2 days, whereas cultures inoculated with samples containing fewer viable spores took 3 to 6 days before reaching high density. Sequential cycles of vegetative growth in liquid culture followed by 5 days of development on TPM agar were repeated over five or six cycles. Alternating development and growth phases are termed 'd1', 'g1', 'd2', 'g2', etc. in temporal order.

(iii) *Dilution platings*

The sizes of both competitor subpopulations within each competition population were estimated at the end of each developmental phase and the end of each growth phase. After development, the 500 µl of sample remaining (after transfer of 500 µl to CTT liquid) was sonicated by means of a microtip and diluted into two distinct selective agar media (or selective and non-selective agar in mixes with W1). At the end of growth in liquid culture, just before centrifugation, samples from liquid cultures were diluted in TPM liquid. The diluted samples (multiple dilutions) were mixed with 10 ml melted CTT soft (0.5%) agar (cooled to 45 °C) containing rifampicin at 5 µg ml^{-1}, kanamycin at 40 µg ml^{-1}, or oxytetracycline at 12.5 µg ml^{-1}, and plated. In all competition mixes with a cheater, competing genotypes were resistant to distinct antibiotics and diluted samples were plated into agar medium containing each antibiotic separately. Population size estimates for each strain were then calculated from plate counts from the appropriate antibiotic treatment, adjusted by the relevant dilution factor.

In most cases, competitions were conducted in two temporally independent experimental blocks. (Exceptions were mixes C1/W1 (one block) and W2/C3 (three blocks).) Each pairwise competition was replicated at least three times within each temporal block.

3. RESULTS

(a) *Wild-type control competitions*

Competitions between marked and unmarked clones of the wild-type strain were performed as controls to compare the population dynamics of wild-type-only mixes with the dynamics of mixes in which cheaters were present. In these control experiments, total spore production remained consistently high in all developmental cycles (figure 2). Wild-type strain W3 showed a slight overall advantage over W1, gradually increasing in frequency over several cycles of development (figure 2*a*), whereas strain W2 gradually decreased in frequency (figure 2*b*). This latter result is consistent with those of previous development assays of W1 and W2 in isolation in which W2 has exhibited slightly lower sporulation levels than W1 (Velicer *et al.* 1998, 2002; F. Fiegna and G. J. Velicer, unpublished data). Total spore production and fruiting body quality remained consistently high throughout all competition cycles.

(b) *Non-disruptive cheater maintenance (C1)*

Cheater C1 was maintained in a balance in all of its competitions with W3 (e.g. figure 3). Despite its massive sporulation defect in pure culture, C1 caused no major disruptions of total population dynamics relative to the

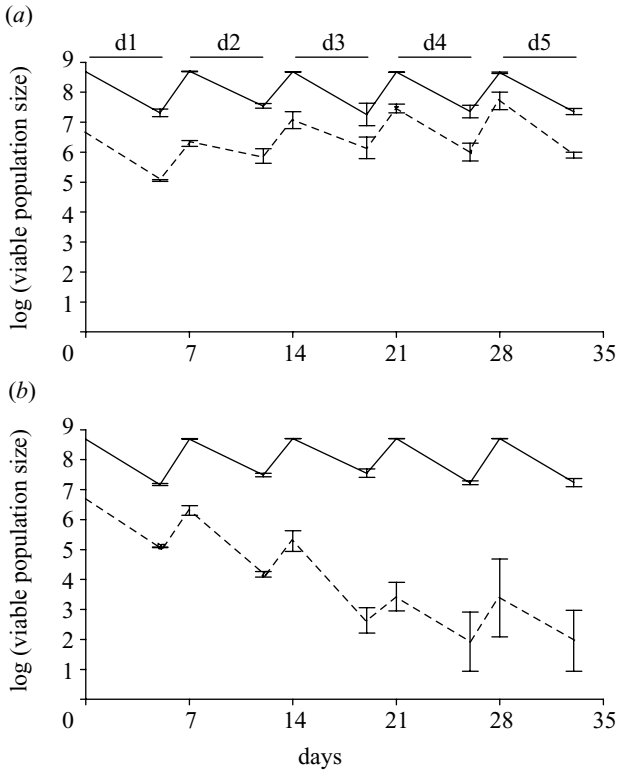

Figure 2. Competition dynamics between unmarked (solid lines, W1) and genetically marked (dashed lines (*a*) W3 and (*b*) W2) wild-type strains that are all proficient at spore production in isolation. During development phases (e.g. d1, d2, etc.) on starvation plates, the total viable population size decreased, whereas total population size increases occurred during growth phases in liquid culture. The total population size entering each development phase is 5×10^8 cells in all figures. Data points show mean population sizes across three independent competition replicates from a single experimental block. Error bars indicate 95% confidence intervals.

control competitions in either experimental block (figure 2). Although C1 reached frequencies equal to and even greater than W3 at the onset of a developmental phase in several cases, its presence at these relatively high frequencies had little effect on W3 or total spore production. In figure 3, note that C1 gained in relative frequency during all growth phases, but showed superior sporulation efficiency only during d1, where its initial frequency was low. During all subsequent developmental phases, C1 began development at much higher frequencies and showed inferior sporulation to W3.

We also tested the fate of C1 in a competition where it began as the dominant genotype (99%) and W3 was in the minority (1%). In three out of four replicates, the high cheater frequency caused total extinction (data not shown), with no viable spores surviving d1 (first developmental phase). In the fourth replicate, a small number of spores (below our detection limit of approximately 10) of both competing genotypes survived and grew to high density after the initial starvation phase. Over subsequent selection cycles, the cheater in this replicate was maintained at frequencies and dynamics similar to those shown in figure 3. Thus, C1 did not reach a disruptively high frequency of its own accord, although its frequency can be directly manipulated to disruptive levels.

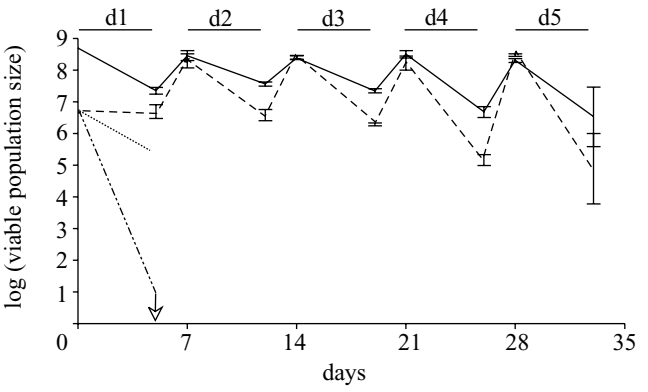

Figure 3. Non-disruptive persistence of cheater C1 (dashed line) in the presence of wild-type W3 (solid line). The dotted line during d1 shows the expectation for C1 under the hypothesis that, as a one per cent minority in mixture with W3, it sporulates with wild-type efficiency (H2 from Velicer *et al.* 2000). The dash-dotted line shows the predicted spore production of C1 (below our limit of detection, arrow) as the minority if it sporulates with the same efficiency in mixture as it does in isolated pure culture (H1 from Velicer *et al.* 2000). Cheating occurs when a developmentally defective strain exceeds the expectation under H2, where the relative frequency of a minority genotype does not change during development. Here, a cheating phenotype is seen only during d1, where the frequency of C1 relative to W3 increases, but not during d2–d5, where its frequency decreases in each case. Data points and errors bars have the same symbolism as in figure 2.

(c) *Disruptive cheater persistence (C2)*

Mixes with manipulated relative frequencies of W3 and C2 in a previous study indicated that C2 sporulates more efficiently than W3 when C2 is rare, but less efficiently than W3 when C2 is common (Velicer *et al.* 2000). This chicken game scenario predicts that during extended competition over multiple developmental cycles, these two genotypes should be maintained together in a balanced polymorphism. It was also observed that C2 causes large decreases in total spore production at frequencies above *ca.* 0.32 (Velicer *et al.* 2000). We could therefore also predict that C2 should cause observable fluctuations in total spore production if it can rise to a sufficiently high frequency.

The results of all replicate competitions of W3/C2 that were conducted (eight in total, in two blocks) support both of the above predictions. In all cases, both the cheater and wild-type genotypes persisted, but only after significant drops in total population size (relative to pure wild-type controls) during one or more developmental phases (e.g. figure 4). All cases of large total population decreases during development (below the levels in wild-type controls) were preceded by the cheater genotype having attained a high relative frequency at the onset of the relevant developmental phase. During such population crashes, C2 always decreased in relative frequency owing to its lower sporulation efficiency than W3. In some replicates, such as the one shown in figure 4*a*, C2 populations fell very close to extinction during d2. Fruiting body aggregates were either small or non-existent during instances of development in which total spore production was very low (e.g. d2 in figure 4*a*), in contrast to normal fruiting bodies when W3 was in a large majority. Across

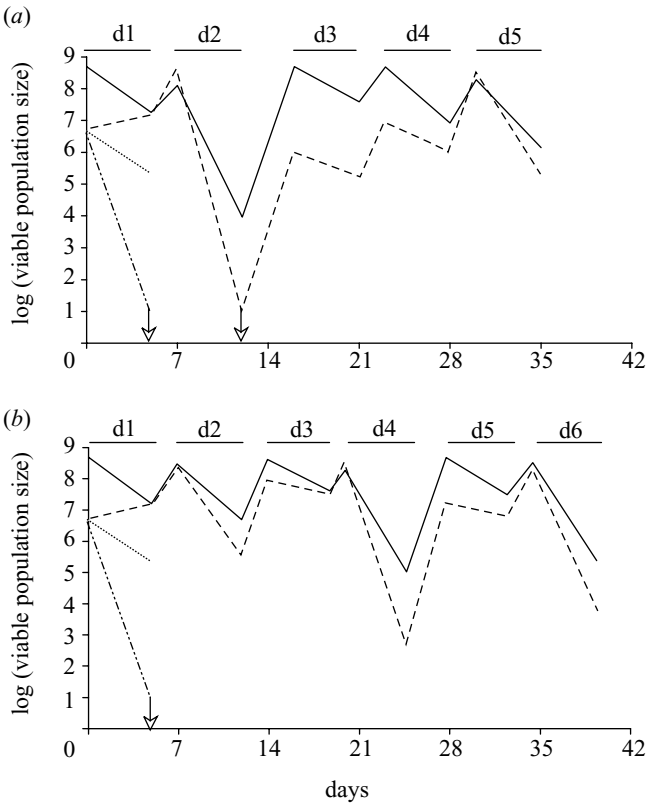

Figure 4. Disruptive persistence of cheater C2 (dashed lines) in the presence of wild-type W3 (solid lines). The data shown are from single replicate competitions from (*a*) the first and (*b*) second blocks of W3/C2 competition experiments. Dotted and dash-dotted lines, and arrows, have the same symbolism as in figure 3.

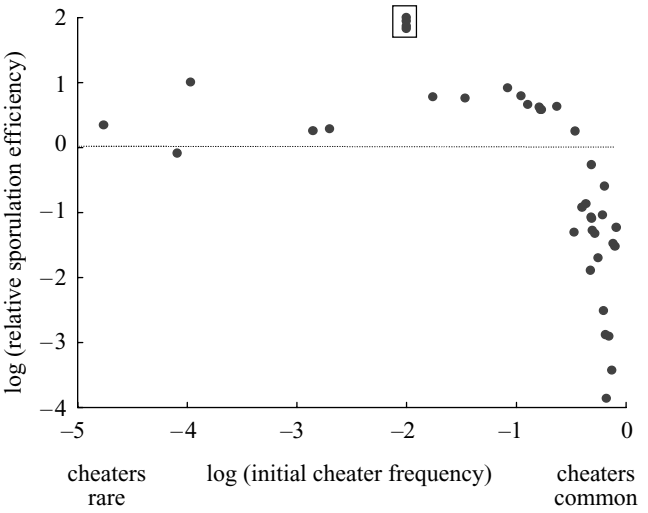

Figure 5. Sporulation efficiency of cheater C2 relative to W3. The dotted line indicates the level of equal efficiency between the two strains. The open box encompasses the eight values from first-round (d1) development assays. Sporulation efficiency was calculated as the ratio of spores produced by either strain in a given round of development to that strain's respective population size at the onset of development. Relative sporulation efficiency is the ratio of C2 efficiency over W3 efficiency.

all replicates and cycles, when C2 entered starvation at frequencies above 0.1, total spore production (W3 and C2 combined) showed a strong negative correlation with C2 frequency ($r = -0.727$, $p < 0.001$, linear regression with log-transformed data).

When C2 entered starvation at frequencies lower than 0.38, it sporulated more efficiently than W3 in 22/24 cases (figure 5). At C2 frequencies above 0.38, however, W3 was more efficient in all 19 cases. These data are consistent with previous results observed after manipulating mixing frequencies, where the C2 frequency at which C2 and W3 sporulate with equal efficiency was estimated to be *ca.* 0.42 (Velicer *et al.* 2000). They are also consistent with the prediction that C2 and W3 should be maintained in a balanced polymorphism.

Interestingly, cheating was much more pronounced during d1 than in any subsequent phase of development. This was the case even when the C2 frequency at starvation onset was lower than in d1, where the C2 frequency was always 0.01 (figure 5). Across all eight W3/C2 competition replicates, C2 sporulated on average 102-fold more efficiently during d1 than did W3, whereas C2 was only 4.6-fold more efficient than W3 in all 14 instances of cheating in later developmental phases. This large drop in cheating efficiency may be a result of an undefined physiological effect in populations having recently undergone the stress of prolonged starvation, which occurred before all developmental phases except d1.

(d) *Total extinction and cheater self-extinction (C3)*

In two independently initiated experimental blocks, extinction events were observed in all replicate competitions (four per block) between W2 and C3 (e.g. figure 6). In both blocks, the C3 subpopulations attained majority status in all replicates by the onset of d2 (average frequencies of 0.87 and 0.89 in the first and second blocks, respectively). The low relative frequency of W2 cells resulted in extremely low or zero viable spore production by the mixed populations during d2. In two replicates of each block, no viable spores survived into g2 (second growth phase), resulting in total population extinction (e.g. figure 6*a*). No growth of either genotype was detected in the g2 flasks of these replicates over a three-week period (during which water was periodically added to prevent culture desiccation). In the remaining two replicates from each block, spores of W2, but not of C3, survived d2, resulting in pure W2 cultures during g2 (e.g. figure 6*b*). No C3 colonies were observed from undiluted samples plated in kanamycin-agar throughout all subsequent competition cycles. The surviving pure W2 populations sporulated at wild-type levels in all subsequent developmental phases.

A third experimental block with eight W2/C3 replicate competitions was also initiated. In all replicates, total spore production in d2 was reduced by high cheater frequencies, but no extinction events took place in any replicate. This different result in the third block is probably a result of lower relative frequencies of C3 at the onset of d2 (mean frequency of 0.41) in the third block than in the first and second blocks (0.87 and 0.89, respectively). This interpretation is consistent with previous results from manipulated W2/C3 mixes where C3 was present at an initial frequency of 0.5 and total spore production was only slightly lower than in pure wild-type controls (Velicer

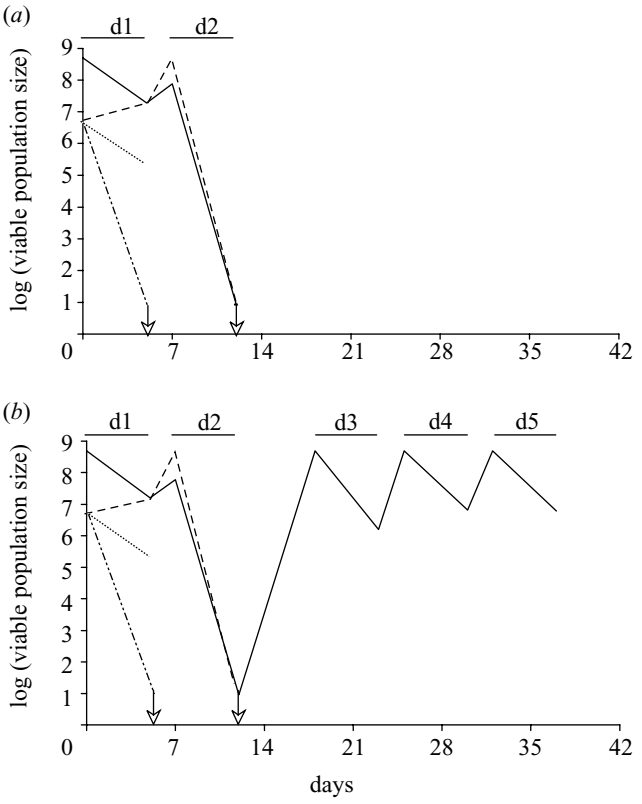

Figure 6. (*a*) Total extinction and (*b*) cheater-only extinction in independent replicate competitions between cheater C3 (dashed lines) and wild-type W2 (solid lines). Dotted and dash-dotted lines, and arrows, have the same symbolism as in figure 3.

et al. 2002). During subsequent developmental cycles (d3–d5), large population crashes (but no extinction events) eventually occurred in each replicate. Overall, eight out of 16 total replicates from three experimental blocks incurred extinction events.

4. DISCUSSION

Obligate social cheaters that do not drive either their altruistic host or themselves to extinction have the potential to persist over extended periods in the absence of effective policing by the host (Matapurkar & Watve 1997). Oscillation dynamics for the relative frequency of cheaters and altruists and the total size of mixed populations should be primarily a function of the relationship between cheater frequency and cheater fitness. In a previous study, cheating behaviour by several *M. xanthus* genotypes was observed during a single round of development (Velicer *et al.* 2000). It was shown that manipulated elevations of initial cheater frequency could cause major decreases in total population spore production and reverse the relative fitness superiority of competing altruistic and cheating genotypes.

Here, we have shown that two distinct cheater genotypes (C2 and C3) both rise, without experimental manipulation, to frequencies high enough to cause large population disruptions over sequential cycles of *M. xanthus* development. A third cheater (C1) caused almost no disruption of total population dynamics, despite being defective at spore production in pure culture. These intense and highly self-defective cheaters show a range of competitive fates, including self-extinction, total extinction of both cheater and altruist, and coexistence with oscillating relative frequency and total population size. The variation between strains in cheater effects on total population dynamics is most likely a result of quantitative differences between the strains in the relationship between cheater frequency and total spore production.

When cheaters are common in this *M. xanthus* system, defection from cooperation is detrimental not only at the individual level (altruists, when rare, beat cheaters) and the group level (fruiting bodies are defective), but also at the population level, where entire populations can approach or suffer extinction. Because small populations are more likely to face extinction as a result of stochastic events than are large ones, any ecological forces that cause major population decreases, including maladaptive social structures, increase the probability of local extinction events. For example, populations of cooperatively breeding animals, such as mongooses and African wild dogs, are especially threatened by small population sizes owing to decreased offspring survival at low densities (Clutton-Brock *et al.* 1999; Courchamp & MacDonald 2001; Dennis 2002). *Myxococcus xanthus* shares this heightened susceptibility to extinction at low densities as a result of the existence of a minimum population density necessary to induce fruiting body formation (Kuspa *et al.* 1992). Low genetic diversity can also increase extinction risk by decreasing adaptability to changing environments (Keller & Waller 2002). In sexual social organisms, some social mating structures can decrease effective population sizes, thereby degrading the maintenance of genetic diversity over time and increasing the risk of extinction (Alberts *et al.* 2002). Here, we provide direct experimental evidence that non-manipulated changes in the social structure of *M. xanthus* populations can lead to major population disruptions and local extinction events.

Our results graphically illustrate the potential for individual-level selection to harm the fitness of cooperative groups (fruiting bodies) and whole populations of such groups. Although our cheater genotypes were derived experimentally, a conceptual analogy can nonetheless be made between the independent competition replicates described here and geographically distinct natural populations of *M. xanthus*. In nature, large population decreases induced by high cheater frequencies would increase the probability of local populations undergoing extinction. Such local extinction events would open previously occupied habitat patches to colonization by any neighbouring populations that were not eliminated by the harmful effects of developmentally defective cheaters. Even without outright extinction events, cheater-induced population decreases would make the victimized population more susceptible to invasion by any neighbouring populations less burdened by cheater parasitism (Sturtevant 1938). The extent of developmental cheating in natural populations of *M. xanthus* and the population-level effects (if any) of such cheating have yet to be investigated.

The degree to which evolutionarily obligate cheaters decrease the total spore production of an *M. xanthus* population over a defined period (relative to a similar population with no cheaters) can be termed the 'cheating

load' of that population (Velicer 2003). More generally, the concept of cheating load is applicable to any population of organisms where individuals that engage in cooperative behaviour are plagued by cheaters that endanger group survival by following evolutionarily selfish strategies. Obligate social parasites that cheat effectively without imposing a large cheating load on host populations are more likely to persist in nature than are more disruptive cheaters.

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