Social interactions among diverse individuals that encounter one another in nature have often been studied among animals but rarely among microbes. For example, evolutionary forces that determine natural frequencies of bacteria that express cooperative behaviors at low levels remain poorly understood. Natural isolates of the soil bacterium *Myxococcus xanthus* sampled from the same fruiting body often vary in social phenotypes, such as group swarming and multicellular development. Here, we tested whether genotypes highly proficient at swarming or development might promote the persistence of less socially proficient genotypes from the same fruiting body. Fast-swarming strains complemented slower isolates, allowing the latter to keep pace with faster strains in mixed groups. During development, one low-sporulating strain was antagonized by high sporulators, whereas others with severe developmental defects had those defects partially complemented by high-sporulating strains. Despite declining in frequency overall during competition experiments spanning multiple cycles of development, developmentally defective strains exhibited advantages during the growth phases of competitions. These results suggest that microbes with low-sociality phenotypes often benefit from interacting with more socially proficient strains. Such complementation may combine with advantages at other traits to increase equilibrium frequencies of low-sociality genotypes in natural populations.

1. Introduction

Animal societies are highly diverse, with many instances of within-group diversity appearing to be functionally beneficial at the group level [1,2]. For example, division of labor among distinct classes of social animals is often thought to improve group productivity [3,4]. However, less is known about the extent and selective character of social diversity within natural groups of microbes, which are increasingly being used in studies of social evolution owing to their relative genetic simplicity and experimental tractability [5–13].

Natural diversity in the degree to which cooperative traits are expressed by distinct strains within a common social group has been found or postulated to exist in several microbial species [14–17]. Examples include variation in quorum-sensing signal production in *Pseudomonas aeruginosa* [18], bioluminescence in *Vibrio fisheri* [19], and social swarming [20] and spore production [21] in *Myxococcus xanthus*. However, little is understood regarding how the evolutionary forces of mutation, selection and genetic drift dynamically shape observed forms and levels of such diversity across heterogeneous environments [18,19,22–25]. Among other factors, interactions between microbial genotypes that differ in the degree to which they express a social phenotype are likely to influence diversity patterns both within and across groups of interacting individuals.

One type of interaction that can alter allele frequencies is social complementation, in which high expression of a focal social trait by one genotype increases the fitness of another genotype that expresses the trait to a lower degree. Such complementation might result in a cheating phenotype in which such ‘low-sociality’
genotypes have higher relative fitness than ‘high-sociality’ genotypes in mixed groups, despite having lower relative fitness in pure groups [26]. Cheating has been demonstrated in a number of microbial systems under laboratory conditions [7,27–29], but the degree to which cheating occurs in natural populations of microbes remains largely unclear [18]. Alternatively, complementation of low-sociality phenotypes might only be partial such that the fitness benefit conferred to them from high-sociality genotypes is insufficient to grant them a relative fitness advantage over the high-sociality genotypes with which they interact [27]. In this study, we performed laboratory experiments to investigate whether social complementation is likely to affect levels of social diversity present within natural social groups of the cooperative bacterium Myxococcus xanthus.

The Gram-negative myxobacteria, including M. xanthus, dwell in terrestrial soils worldwide [30] and exhibit complex suites of social traits. For example, although M. xanthus cells are individually motile [31], they also engage in social motility (S-motility) mediated by Type-IV pili and other extracellular matrix components [32,33]. Myxococcus xanthus cells prey upon other microbes in the soil by secreting lytic enzymes that convert their prey into usable growth substrates [34,35] in a manner hypothesized to be density dependent [36]. Finally, when M. xanthus cells deplete local growth resources, a complex group response is triggered that results in the formation of multicellular fruiting bodies, in which a minority of cells convert into stress-resistant spores [37–39].

Myxococcus xanthus social phenotypes are highly variable among clones isolated from soil at both large and small spatial scales [11,20,21,40], and can undergo rapid evolutionary change in experimental populations [5,13,41]. Naturally variable traits include developmental competitiveness in mixed groups [11,27,42], social swarming rates [20], rate of development [21], allelic effects during development [43] and predation range [40]. Kraemer & Velicer [14] found that even closely related clones isolated from within a single fruiting body often vary markedly in their expression of social phenotypes. Three of 10 fruiting bodies they examined harboured genotypes that swarm significantly slower in pure culture than do other clones from the same fruiting body. Moreover, five of the 10 fruiting bodies contained minority genotypes with significantly lower pure-culture sporulation efficiencies (approx. 0–90%) than the dominant clones in their respective fruiting body. In all cases, the isolates exhibiting low social performance were observed at frequencies of at least 0.02 among samples from the same fruiting body (n = 48).

The presence of genotypes with distinct social phenotypes in natural kin group opens up the possibility that within-group interactions substantially influence genotype frequencies in the wild and might drive coevolutionary dynamics.

In this study, we examined whether natural isolates that are relatively poor at group swarming or multicellular development in pure culture are complemented by other strains isolated from the same fruiting body, and whether any such complementation generates a relative fitness advantage for the low-sociality strains (i.e., cheating). For group motility, we asked whether fast genotypes promote faster swarming of slower genotypes in mixed groups relative to pure culture. For multicellular development, we asked whether the presence of a high-sporulating strain in a mixed culture increases the sporulation efficiency of strains that sporulate very poorly or not at all in pure culture. We also tested whether two low-sporulation strains that are complemented, but only partially, by high-sporulation strains during development have growth-rate advantages that might offset their relative disadvantage during development.

2. Material and methods

(a) Clones

The M. xanthus clones examined here were all isolated from three individual fruiting bodies that emerged on soil collected from the Indiana University Research and Teaching Preserves at Moores Creek and Kent Farm near Bloomington, Indiana, USA [14]. Briefly, individual M. xanthus fruiting bodies were picked from soil samples, and non-sporule cells were killed by heat and sonication. Subsequently, multiple independent clonal isolates were derived from the surviving spore population of each fruiting body, stored frozen, and screened for phenotypic and genotypic differences [14]. In this study, we examined subsets of the original clone sets isolated from each fruiting body. These subsets include clones that exhibited significant within-fruiting-body variation in swarming rate, spore production or both. Social trait phenotypes vary continuously in natural populations of M. xanthus [14,20,21,40–43]. Nonetheless, we found previously that subsets of isolates samples from a single fruiting body often cluster, apparently discretely, within significantly different social phenotype ranges in a manner detectable by a k-means cluster algorithm [14]. We refer to genotypes as fast or slow swarmer, or high or low sporulators, relative to one another in a manner that is empirically grounded and is consistent with social phenotype values among larger sample sets varying continuously.

Clones were marked with either rifampicin or kanamycin resistance to allow detection and population size estimates of distinct genotypes in mixed cultures. We selected for spontaneous rifampicin-resistant mutants and kanamycin-resistant transformants carrying the plasmid pREG1727 [44]. Only marked clones that exhibited no significant marker effects on focal traits relative to their unmarked parent were used for further experiments (data available upon request). When possible, interaction assays were performed with reciprocal pairings of marked and unmarked clones. Table S1 in the electronic supplementary material lists all marked clones, their swarming or sporulation phenotype relative to the unmarked majority clone type from the same fruiting body, and antibiotic resistance state.

(b) Interactions during social swarming

Clones to be tested for interactions during swarming were grown up from frozen stocks into 8 ml Casitone-Tris (CTT) liquid medium [45] until turbid. Cultures that reached exponential growth phase before others were diluted with fresh medium to avoid entry into stationary phase. The day prior to the assay, all cultures were diluted to 3 × 10^7 cells ml^-1 with fresh medium. Swarming assay plates were prepared 1 day prior to assay initiation by pouring 25 ml of CTT soft (0.8%) agar into 9 cm Petri dishes and allowing them to solidify and dry in a laminar flow hood without lids for 15–20 min, after which they were capped and stored overnight at room temperature.

To initiate swarming assays, 5 ml of exponential phase culture of each clone were spun down for 15 min at 4500g and resuspended with CTT liquid medium to 5 × 10^7 cells ml^-1. Combinations of marked and unmarked clones were mixed in a 9:1 ratio. Ten microlitres of these mixed cultures (or of pure-culture controls) were spotted onto the centre of the prepared plates and dried in a laminar flow hood. After 1 and 5 days of incubation at 32°C, 90% rH, the swarm perimeter was marked. Swarming rate was calculated as the average distance moved by the swarm edge across at least four perpendicular vectors at random orientation.

To test whether cells marked with antibiotic resistance were present at the leading edge of swarming populations, we
randomly picked 10 samples from each swarm edge from locations roughly evenly distributed around its perimeter with a sterile pipette tip. These samples were streaked onto CTT hard (1.5%) agar plates containing 5 μg ml⁻¹ rifampicin, as well as onto hard agar plates containing no antibiotics, and the presence or absence of growth was scored after 3–4 days of incubation.

(c) Interactions during development
Clones were cultured as described for swarming until the initiation of swarming assays. Development plates were prepared by pouring 8 ml of TPM starvation hard (1.5%) agar containing no added carbon substrate [38] into small Petri dishes. On the day of the assay, liquid cultures were spun down for 15 min at 4500g and resuspended to a density of 5 × 10⁸ cells ml⁻¹ with TPM liquid medium. For direct competition treatments, the unmarked clone of one competitor was mixed with the marked version of the other competitor at a 9 : 1 ratio and 10 μl of the mixture was spotted onto the centre of a TPM agar plate. As controls, 10 μl of pure culture was spotted on separate plates for each competitor. The plates were subsequently incubated at 32°C, 90% rH for 3 days, as were all plate cultures described subsequently. After this period, starving cultures were harvested with a sterile scalpel blade and transferred into 1 ml sterile ddH₂O. These spore samples were heated for 2 h, followed by sonication twice for 10 s to destroy all non-spore cells.

For experiments lasting only one developmental cycle, the spore sample was diluted into CTT soft agar cooled to 45°C with and without antibiotic. Plates were incubated for one week at 32°C, 90% rH before colonies were counted. For assays carried out over five developmental cycles, half of the spore sample was transferred into 8 ml fresh CTT liquid medium and incubated at 32°C, 300 r.p.m. Cultures grew up after 3–6 days, were standardized to a density of 5 × 10⁸ cells ml⁻¹ in TPM liquid after centrifugation and were used to initiate the next cycle of development. This was repeated for a total of five cycles of development and growth. The remainder of the spore sample was used for dilution into CTT soft agar, as described above. All assays were performed in at least three temporally independent replicate blocks, both for this experiment and all others.

(d) Statistics
We performed all statistical tests using R [46]. To investigate swarming interactions, we compared the distance moved per day by mixed versus pure swarms and tested for a deviation of the difference between these values from zero using one-sample one-tailed t-tests.

For all developmental assays, spore counts plus one were log₁₀-transformed before analysis. We tested for partial or full complementation by comparing the actual spore production of low-sporulation strains in mixed competitions relative to expectations under two hypotheses: the H₀ hypothesis that the sporation efficiency of low-sporulation strains is unaffected by mixing with high-sporulation strains, and the H₁ hypothesis that mixing of high- and low-sporulation strains increased the sporation efficiency of the latter to the level of the former. For both hypotheses, we tested whether the mean per-mix difference between actual and expected spore counts (calculated for each mixed pair of strains within each of three or four independent replicate blocks) differed significantly from zero using one-sample two-tailed Wilcoxon exact rank-sum tests (except for the H₀ test of clone c29K, in which case a negative deviation from the expected value was impossible and a one-sample one-tailed Wilcoxon exact rank-sum test was used).

Frequency trajectories of low-sporulation clones during multiple rounds of growth and development were determined by calculating the slope for each replicate based on the logarithm of low sporulator frequencies at the end of each growth and developmental cycle, and testing for a negative deviation of these slopes from zero using two-tailed one-sample t-tests. All p-values were corrected for multiple comparisons performed within each dataset with the Hochberg method [47].

Figure 1. Slow swarming is complemented by fast swimmers in mixed groups composed of clones from fruiting body KF3.2.8. (a) Pure culture swarming rates. (b) Swarming rates (mm day⁻¹) of mixed swarms of fast clones c11 and c30 each initially mixed with the slow clone c32 at both 9 : 1 and 1 : 9 ratios. Solid and dashed lines indicate the pure culture swarming rates of the fast clone present in each mixture and of the slow clone c32, respectively (also shown in (a)). p-values near the solid and dashed lines reflect tests for differences between mixed group swarming rates versus the pure culture rates of the fast and slow clone in each mix, respectively. 'n.s.', non-significant; *p < 0.05. Error bars represent 95% CI.

(e) Semantics
The social semantics employed here follow previous usage [11,13,14,26,27,29,41,42,48,49]. 'Complementation' refers to a positive effect of a high-performance genotype on the absolute fitness of a low-performance genotype, regardless of relative fitness. Inversely, 'exploitation' by a low-performance strain refers to the strain's ability to be so complemented, again regardless of relative fitness. In other contexts, 'exploitation' also applies more broadly to any gain in absolute fitness via social interaction [11,42]. 'Cheating' also refers to a specific fitness relationship, namely exploitation of a strain with high performance at a focal social trait in pure culture by a low-performance strain that confers a relative within-group fitness advantage to the low-performance strain. These definitions are not contingent on the molecular, behavioural or evolutionary causes of the respective phenotypes, which may not be known.

3. Results
(a) Social complementation during group swarming
The KF3.2.8 fruiting body was composed of clones that can be grouped into fast and slow S-motility classes, with the slower class being the majority [14]. We tested whether a slow clone (c32) might socially exploit fast clones (c11 and c30) in a manner allowing it to swarm faster in their presence than in pure culture (figure 1). All clones were marked with rifampicin resistance, and each fast clone was mixed with c32 at both 9 : 1 and 1 : 9 initial ratios. Mixed populations were allowed to swarm outward for 5 days, after which the mixed group swarming rates were compared with those of each genotype in pure culture and the leading edges of mixed swarms were tested for the presence of c32.
In mixed cultures, the presence of c32 did not significantly reduce the overall group swarming rate relative to pure cultures of the fast clones (c11 and c30, figure 1) if it started out in the minority (electronic supplementary material, table S2; one-tailed one-sample t-test, all \( p > 0.05 \)). When the slow clone was initially in the majority, swarming rates were reduced relative to pure cultures of fast clones, but significantly so only in c32 : c30 mixes (figure 1; electronic supplementary material, table S2; one-tailed one-sample t-tests, c32 : c11: \( p = 0.083 \); c32 : c30: \( p = 0.031 \)).

All mixed swarms moved significantly faster than pure cultures of c32 alone (figure 1; electronic supplementary material, table S2; one-tailed one-sample t-tests, all \( p < 0.05 \)). Importantly, this result raised the possibility that c32 might swarm faster in the presence of fast clones than in pure culture. To test this hypothesis, swarm-edge samples from these mixed culture competitions were taken at distances from the initially inoculated area greater than those reached by pure c32 cultures. All of these samples (10 per replicate) tested positive for the presence of c32, thus demonstrating social complementation of the slower clone during group swarming.

(b) Social complementation during development

For two fruiting bodies (MC3.3.5, MC3.5.9), low-sporulating clones marked with antibiotic resistance were mixed pairwise at a 1 : 9 ratio with high-sporulating isolates from the same fruiting body. We tested whether the presence of high-sporulating strains affected the absolute fitness of paired low sporulators positively (social exploitation and complementation by the low- and high-sporulating strains, respectively), negatively (suppression) or not at all (null hypothesis, \( H_0 \)). For instances of social exploitation, we tested whether the relative fitness of low sporulators was higher than, equal to (hypothesis \( H_1 \)) or lower than that of paired high sporulators (cheating, full complementation or partial complementation, respectively; electronic supplementary material, table S3).

(i) Fruiting body MC3.3.5

Clones from fruiting body group MC3.3.5 were previously found to vary in pure culture spore production, with two clones sporulating at a similarly low level relative to five other clones [14]. In pure culture, the low-sporulating clone that we examine here, clone c8, produces approximately 16–42% of the number of spores produced by the four high sporulators examined here (c9, c21, c34, c44; figure 2a [14]). Surprisingly, mixing c8 with the high sporulators appears to have reduced c8 sporulation rather than complementing it; c8 spore estimates were lower than expected under \( H_0 \), in mixes with all four high-sporulating strains, but not significantly so (figure 2b; Wilcoxon two-tailed exact rank-sum test of deviation of the average performance of c8 mixed with each high sporulator from \( H_0 \): \( p = 0.125 \); electronic supplementary material, table S3). When mixed with high sporulators, c8 produced on average 81% fewer spores than expected based on pure culture assays (average sporulation efficiency with c9: 9%, c21: 32%, c34: 34%, c44: 10% of the \( H_0 \) expectation). Mixing of c8 with high sporulators did not significantly impact total group performance in comparison with pure cultures (figure 2).

(ii) Fruiting body MC3.5.9

The MC3.5.9 fruiting body also contained both high- (c5, c18, c25, c31, c44 and c46) and low- (c22, c29, c36 and c39) sporulating isolates. In our experiments, two low sporulators (c22 and c36) produced a significant number of spores in pure culture, whereas the other two (c29 and c39) did not (figure 3a). We marked the completely defective isolates c29 and c39 with antibiotic resistance and tested their performance in 1 : 9...
mixtures with both high-sporulating clones and other low sporulators that produce some spores in pure culture (electronic supplementary material, table S3; figure 3). In almost all mixes, the pure culture sporulation defects of c29 and c39 were partially complemented by the high-sporulating clones (figure 3b, c; Wilcoxon exact rank-sum tests, in all cases $p$, 0.05 for negative deviation from H1 and positive deviation from H0; electronic supplementary material, table S3). Because complementation was partial, absolute fitness of c29 and c39 was increased by the high-sporulation clones, but cheating did not occur (i.e. relative fitness of the low-sporulation strains was always lower than 1.0; figure 3b, c; electronic supplementary material, table S3). Neither c29 nor c39 was complemented by any other low-sporulation clone (figure 3b, c).

**Figure 3.** High-sporulating clones from group MC3.5.9 partially complement sporulation by the developmentally defective clones c29K and c39K. (a) Pure-culture sporulation by clones isolated from fruiting body MC3.5.9 and antibiotic resistant variants c29K and c39K. (b) Total group (grey bars) and c29K (white bars) sporulation in mixtures of c29K with other clones from MC3.5.9. (c) Total group (grey bars) and c39K (white bars) sporulation in mixtures of c39K with other clones from MC3.5.9. Solid lines indicate expected sporulation by c29K or c39K under the H1 hypothesis that the high-sporulating strain in each mix increases the sporulation efficiency of the poor sporulator to its own level. The dashed lines show expected c29K or c39K performance under the H0 hypothesis that the sporulation efficiency of the respective poor sporulator is unaffected by mixing. ($H_0 = 0$ for c29K but the dashed line is placed slightly above zero for visibility.) Error bars represent 95% CIs.

**Competitions over multiple cycles of development and growth**

We tested whether the presence of the developmentally defective isolates c29 and c39 in group MC3.5.9 might be owing to a faster germination and/or vegetative growth rate by competing these strains with the high-sporulating clone c25 over five cycles of growth and development. Experiments were begun at two initial strain ratios (9 : 1 and 1 : 9) and subsequent frequencies were tracked at the end of each growth and development phase (figure 4).

Both c29 and c39 decreased in frequency overall during the course of each competition, regardless of their initial frequency (figure 4; electronic supplementary material, table S4;
two-tailed one-sample $t$-tests of slope deviation from zero for the logarithm of $c_{29}$ or $c_{39}$ frequency over time, all $p < 0.05$ and $n = 3$ in all cases). Nonetheless, despite their overall decrease, both strains persisted longer in these multi-cycle competitions than expected from their developmental performance in pure culture, which predicts that $c_{29}$ should go extinct in the first round of development (because it makes no spores alone) and $c_{39}$ in the second (because it makes very few spores alone; figure 3c). However, both $c_{29}$ and $c_{39}$ persisted to the end or nearly the end of all competition experiments in all replicates (figure 4).

Both $c_{29}$ and $c_{39}$ exhibited higher fitness during growth phases of the multi-cycle competitions (which include both spore germination and vegetative growth) than $c_{25}$, as they consistently increased in frequency during growth phase (figure 4). Nonetheless, the gains of $c_{29}$ and $c_{39}$ during growth did not outweigh their losses during development, during which they consistently decreased in frequency (figure 4). In summary, although the low-sporulating clones decreased in these experiments overall, both partial social complementation during development and faster germination and/or growth promoted their persistence in our experiments.

Figure 4. Log-transformed frequencies of developmentally deficient clones MC3.5.9 $c_{29}K$ and $c_{39}K$ at the end of each of five consecutive cycles of growth (G) and development (D) in mixes with the high-sporulating clone $c_{25}$. Solid and dashed lines indicate linear regression slopes for each mix. Low-sporulating clones $c_{29}K$ and $c_{39}K$ have an advantage during growth cycles but nonetheless decline in frequency overall owing to their disadvantage during development. $p$-values reflect the significance levels of frequency-slope deviations from zero. (a) Frequencies of $c_{29}K$ in competitions with $c_{25}$ in which $c_{29}K$ began in the minority. (b) Clone $c_{29}K$ frequencies after it began in the majority. (c) Clone $c_{39}K$ frequencies after it began in the minority. (d) Clone $c_{39}K$ frequencies after it began in the majority. Error bars represent 95% CIs.

4. Discussion

In any social species, spontaneous mutation (and recombination in many species) is expected to create diversity in the degree to which cooperative traits are expressed across individuals. Recent studies indicate that such diversity within local natural populations of interacting microbes is common and includes strains that express social traits at a very low level or not at all [14,16,19,50,51]. Understanding the relative contributions of distinct evolutionary forces in determining
the frequencies and fates of genotypes that differ in social trait expression is challenging. It requires understanding the fitness spectra of such variants across the relevant range of abiotic and biotic environments they encounter (including social environments), as well as the environmental and genetic determinants of those spectra [23,24,29]. Among other factors, social interactions between such variants are likely to significantly affect their fitness dynamics.

Here, we have demonstrated that high-sociality genotypes of M. xanthus can have both positive and negative social effects on the fitness of closely related low-sociality genotypes isolated from a common fruiting body. For example, in mixed groups, isolates exhibiting high levels of group swarming or sporulation enhanced the performance of isolates that perform relatively poorly at these traits in pure culture. This fundamental result suggests that social complementation increases the equilibrium frequencies of low-sociality strains in natural populations. However, none of the low-sporulation strains examined here showed a relative fitness advantage over high-sporulation strains in mixed groups owing to social cheating. The degree to which cheating by strains with low-sociality phenotypes occurs in nature and the potential role of cheating in shaping diversity within natural groups of social microbes remain unclear.

(a) Social complementation

Myxococcus swarming rates vary greatly both among and within natural kin groups [14,20]. Previous laboratory experiments had shown that social complementation of relatively poor swarmers by faster swarmers can occur among defined mutants [52], and here we have shown that such complementation can occur among natural social neighbours as well. Slow-swarming isolates swarmed farther when mixed with fast-moving genotypes than when alone and remained present at the leading edge of mixed swarms throughout our competition experiments. These slow strains should have been lost from the leading edge in short order if their swarming were unaltered by the presence of fast strains. Because our experiments only tested for the absolute presence or absence of the slow genotype at the swarm edge and did not estimate strain frequencies, further work is required to resolve whether the complemented slow strains gradually decrease in frequency at the leading edge and would eventually be lost, or rather persist long-term about an equilibrium frequency. If social complementation also occurs in the wild, it would increase the probability that lineages with low-sociality phenotypes may persist long enough to re-evolve self-prociency at the trait at which they are defective [13,52]. Although the complementation observed in our experiments did not confer a cheating advantage to developmentally defective strains, it remains possible that these defective strains do in fact successfully cheat on high-sporulating strains under the very different environmental conditions found in soil, as ecological conditions can strongly influence fitness relationships resulting from social interactions [60,61].

(b) Pleiotropy

Mutations that reduce expression of a social trait might pleiotropically affect fitness in other traits either positively or negatively [5,62]. For example, spontaneous low-sociality mutants of M. xanthus have previously been found to have fitness advantages over high-sociality genotypes in resource-rich laboratory environments in a manner not mediated by social cheating [5,36,41]. Analogously, it has been proposed that strains of P. aeruginosa defective at quorum sensing that are present in cystic fibrosis patients might often have non-social fitness advantages in the lung environment [18], some of which may be caused by the same alleles responsible for low-quorum-sensing phenotypes.

In this study, two low-sporulation isolates exhibited fitness advantages over a high-sporulation strain derived from a common fruiting body during the growth phases of competitions carried out over multiple cycles of development (figure 4).
Although the combination of these growth rate advantages and partial developmental complementation were not sufficient to prevent the low-sociality strains from decreasing in frequency, these factors allowed the defective strains to persist much longer than would have been the case in their absence. Such positive effects of low-sociality mutations on non-social components of fitness are likely to affect the degree and dynamics of social diversity in natural populations as well, and may even be of sufficient magnitude to generate balancing selection that maintains low-sociality genotypes indefinitely.

Some alleles causing low expression of microbial social phenotypes are likely to be net disadvantageous such that their equilibrium frequencies in natural populations are determined by mutation–selection balance [63,64] rather than balancing selection. Other such alleles may be actively maintained by balancing selection mediated by social and/or non-social allelic fitness effects. While our experiments do not resolve between these hypothetical scenarios with respect to the _M. xanthus_ isolates examined here, they do reveal a complex range of fitness relationships among clones that co-resided in a common natural group, including social diversity.

**References**

5. Velicer GJ, Kroos L, Lenski RE. 1998 Loss of co-resided in a common natural group, including social cooperation (figure 2), social complementation (figures 1, 3 and 4) and fitness variation during asocial growth (figure 4). Wilder et al. [18] suggested that multiple distinct selective forces, both social and asocial, are likely to underlie the presence of extensive polymorphism in quorum-sensing expression phenotypes documented within clinical populations of _P. aeruginosa_. Similarly, in addition to the stochastic forces of mutation and drift, we expect that multiple forms of selection shape analogous diversity in social swarming and sporulation phenotypes found within _M. xanthus_ social groups. Proper understanding of such selective forces requires both broader sampling of diversity within natural microbial groups and characterization of fitness relationships among group members across relevant environments.

**Acknowledgements.** We thank past and present members of the Velicer laboratory and two anonymous reviewers for helpful discussions regarding statistical analyses and manuscript preparation. The authors declare no conflict of interest.

**Funding statement.** This study was supported by the National Institutes of Health grant no. GM079690 to G.J.V.