ARTICLES

Evolution of an obligate social cheater to a superior cooperator

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Obligate relationships have evolved many times and can be parasitic or mutualistic. Obligate organisms rely on others to survive and thus coevolve with their host or partner. An important but little explored question is whether obligate status is an evolutionarily terminal condition or whether obligate lineages can evolve back to an autonomous lifestyle. The bacterium *Myxococcus xanthus* survives starvation by the social development of spore-bearing fruiting bodies. Some *M. xanthus* genotypes defective at fruiting body development in isolation can nonetheless exploit proficient genotypes in chimaeric groups. Here we report an evolutionary transition from obligate dependence on an altruistic host to an autonomous mode of social cooperation. This restoration of social independence was caused by a single mutation of large effect that confers fitness superiority over both ancestral genotypes, including immunity from exploitation by the ancestral cheater. Thus, a temporary state of obligate cheating served as an evolutionary stepping-stone to a novel state of autonomous social dominance.

Obligate relationships are common in biology. Higher eukaryotes host a vast array of obligate parasites and mutualists that include both microbes and larger organisms[1–6]. For example, numerous bacteria are obligate pathogens or symbionts[7,8], many bird species are obligate brood parasites[9] and a variety of insects are social parasites that rely on workers of another species to raise their offspring[10–13]. These radically distinct obligate systems share the common theme of dependence on a host or partner (which might be of the same or of a different species) for evolutionary success despite their divergent mechanisms and strategies.

At least in microbes, extensive gene inactivation or loss often occurs during long-term evolution in an obligate state of mutualism or parasitism[7,8], making reversion to free-living status highly unlikely after extended periods of evolution. Although these and other long-term patterns of coevolution in obligate systems have been detected[9,14,15], little is known about the initial stages of obligation in any system[16]. It remains unclear how frequently genetic transitions to obligate status are contingently irreversible[17] or rather might reverse during early stages of obligate coevolution, either directly to the exact ancestral genotype or by a novel mutational pathway[18,19]. In particular, little is known about the potential of obligate social cheaters to evolve new forms of social cooperation that do not depend on the prior social host.

Myxococcus xanthus is the best characterized species of the soil-dwelling myxobacteria, which are distinguished by cooperative predation on other microbes and social development into a wide variety of fruiting body morphologies[20]. Predation is accomplished by swarming packs of cells that secrete toxic and lytic metabolites that kill and degrade prey organisms and thereby generate an extracellular, public pool of resources[21]. Both predation and development are facilitated by two motility systems, one of which is social in nature and employs type-IV pili that mediate cell–cell interactions[22]. Upon amino-acid deprivation, *M. xanthus* cells aggregate into local groups of ~100,000 individuals that exchange intercellular signals to construct spore-bearing fruiting bodies[23]. Importantly, only a minority of cells survive development after differentiation into stress-resistant spores[24], and thus proficiency at competition for limited sporulation 'slots' can be a major fitness component for distinct genotypes undergoing co-development in chimaeric fruiting bodies[25,26]. This microbial system (as well as others[27,28]) readily allows real-time observation of evolutionary changes in social traits[29,30].

A variety of socially exploitative relationships among microbes have been described[25,26,31–34], including developmentally-defective genotypes of *M. xanthus* that are capable of cheating on their developmentally-proficient progenitor during starvation[26,35]. Several such cheaters were isolated from *M. xanthus* populations that had undergone 1,000 generations of evolution in nutrient-rich liquid medium in which positive selection for proficient motility or development was absent[26,30]. Under these conditions all populations incurred major evolutionary losses in social ability, with most lineages becoming defective in both social motility and developmental sporulation.

In mixtures with the developmentally proficient ancestor, however, some of these developmentally incompetent genotypes are able to cheat on their social benefactor by sporulating even more efficiently than the ancestral cooperator[26,35]. One such cheater genotype, here designated 'OC' (Obligate Cheater), fails to produce viable spores in clonal isolation[35]. In this experimental system, OC is thus an obligate social cheater that requires the presence of a developmentally proficient social host (for example, strain GJV2, a marked variant of OC's ancestor GJV1) to avoid extinction during starvation. GJV2 is altruistic in an evolutionary sense because it benefits the fitness of OC to its own competitive disadvantage.

Evolutionary restoration of social autonomy

This obligate cheater (OC) was allowed to compete against the socially competent strain GJV2 over six sequential competition cycles, with each cycle consisting of two phases—development on starvation agar followed immediately by growth in liquid medium[36]. Development and growth phase cycles are hereafter referred to as D1–D6 and G1–G6, respectively. OC began as 1% of the initial mixed population, and the two competitors were marked with resistance to distinct antibiotics (kanamycin for OC and rifampicin for GJV2), which allowed tracking of genotype frequencies throughout the

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competition. In one competition that we focus on here, descendants of OC re-evolved the ability to sporulate independently of GJV2 and overtook the population (Fig. 1).

As a minority in mixed culture with GJV2, the initial OC cheater genotype sporulates more efficiently than GJV2 during development and also shows an advantage during growth in liquid medium[35,36]. Combined, these competitive advantages allowed the OC population to rise rapidly from an initial frequency of 0.01 to majority status (Fig. 1). During the second and third rounds of development (D2 and D3), the increasing frequency of developmentally-defective OC cells led to large reductions in total spore production (of both competitors combined) relative to the first round (D1) in which GJV2 began at a frequency of 0.99. An even more severe sporulation bottleneck occurred during D4, which fewer than 20 individuals (the lower limit of detection) appear to have survived. OC descendants overtook the population after the D4 bottleneck.

If the OC-derived population entering D5 had undergone no evolutionary change since the onset of competition, it should have produced zero or extremely few spores during D5 owing to the developmental defect of OC and the absence of sufficient GJV2 cells necessary to rescue OC sporulation. Surprisingly, the previously defective OC-derived population produced spores at a very high level during D5 and D6, despite the absence of GJV2 cells (Fig. 1). Total spore production during D5 and D6 was 2.5- and 2.8-fold higher, respectively, than during D1 when GJV2 was present as 99% of the population ($P < 0.05$ in both cases, *t*-test).

The radical change in developmental phenotype of the kanamycin-resistant OC-derived population suggested that a spontaneous mutant of OC that had regained sporulation proficiency originated before D4 and survived the D4 bottleneck at high frequency. Nine kanamycin-resistant, rifampicin-sensitive clones (OC-derived) were isolated from culture samples frozen at the end of each growth phase throughout the competition experiment. All OC-derived clones from population samples before D4 (G1–G3) failed to aggregate or produce spores in clonal populations (data not shown). All clones isolated after the D4 crash (G4–G6) showed the positive developmental phenotype exhibited by the original competition population in D5 and D6 (Fig. 1). Hereafter, we refer to the sporulation-proficient genotype derived from OC that arose from the D4 population crash as 'PX' (Phoenix). One PX clone from the G4 population was selected for further analysis. In clonal cultures, PX sporulated sevenfold more efficiently than GJV2 (Fig. 2, $P < 0.01$, *t*-test). We estimate that ≤60 generations of growth occurred between the initial experimental inoculation of OC and the detection of PX.

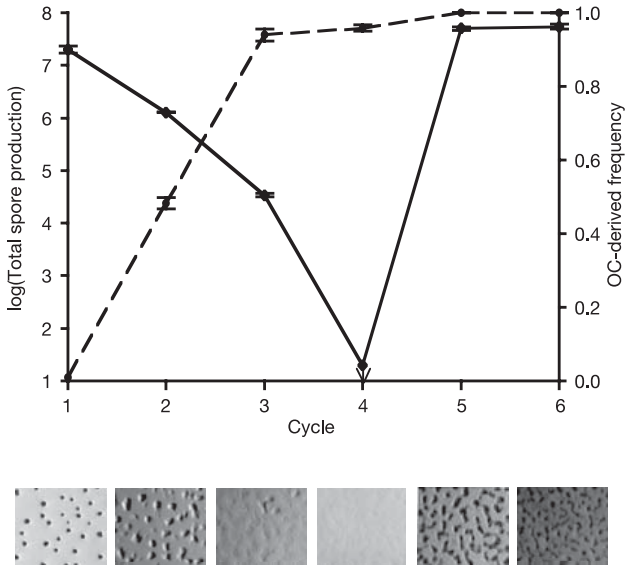

Figure 1 | Total spore production, OC-derived sub-population frequencies and developmental population phenotypes during the original six-cycle competition between OC and GJV2. OC-derived frequencies (dashed line, right axis) represent sub-population frequencies at the beginning of each respective developmental cycle (*x* axis), whereas spore production values (solid line, left axis) represent total spore population sizes at the end of each developmental stage. Developmental phenotypes (images) are shown in sequential order (cycles 1 to 6, left to right). Error bars indicate 95% confidence intervals based on two independent samples of the population at each time point. The arrow indicates that no spores were present at the lower limit of detection in cycle 4.

Usurper dominance

Because OC dominated the mixed OC-derived population at the onset of D4 (frequency >0.89), the very large gain in relative frequency by PX during the fourth cycle of development and growth suggested that PX has a large survival advantage over OC during development when PX is rare and OC is common. This is in fact the case, as the sporulation efficiency of PX is >1,000-fold higher than that of OC in direct competition between these two genotypes when PX is 10% of the initial population (Fig. 3).

The novel PX genotype appears to have fully displaced its immediate ancestor OC as well as the GJV2 population, suggesting that PX is a superior competitor to both. To examine its fitness against these genotypes more thoroughly, PX was mixed at multiple initial frequencies with both competitors independently and its relative sporulation efficiency estimated after one round of development (Fig. 3). PX was developmentally superior to both competitors at all frequencies from 0.1 to 0.9, but showed opposite relationships between frequency and degree of superiority across the two competitors. The advantage of PX over GJV2 increased ~400-fold as PX frequency increased from 0.1 to 0.9, whereas the degree of its advantage over OC decreased ~700-fold over the same frequency range.

The frequency-dependent relationships between OC and the two proficient strains GJV2 and PX were qualitatively similar, with total social productivity (that is, the combined spore production of both competitors in each pairing) and OC fitness (Fig. 3) both being inversely correlated with OC frequency (total sporulation and OC versus GJV2 data not shown). One significant exception is that even at low OC frequencies, extracellular complementation of OC sporulation by PX was only partial (that is, OC gains no cheating advantage against PX from its defect), whereas GJV2 supports a true cheating phenotype in which defection by OC translates into a fitness advantage. Thus, the mutational pathway that restores development in PX also renders PX competitively immune from exploitation by its cheating parent. Such immunity from cheating—or some other form of cheater control[17,37–40]—is necessary for any novel cooperative strategy to succeed when defectors are present.

The result that PX superiority over GJV2 increases dramatically as a positive function of PX frequency suggests that the composition or amount of extracellular compounds that affect development produced by the two genotypes is asymmetric, and that the sporulation efficiency of each strain is greatest when its own molecular profile dominates the extracellular social matrix. Because PX is superior at all frequencies examined, it should rise to dominance in extended

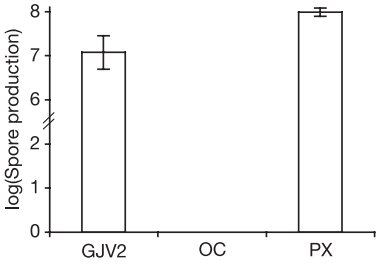

Figure 2 | Pure-culture spore production of strains GJV2, OC and PX. No spores were produced by strain OC. Error bars indicate 95% confidence intervals.

competitions against either competitor over sequential cycles of development. In a subsequent competition with GJV2, PX rapidly rose from 1% of the initial population to near fixation over six rounds of development (Fig. 4).

The developmental superiority of PX over GJV2 does not derive from an ability to sporulate more efficiently in the presence of GJV2 than in isolation (that is, it does not exploit GJV2, data not shown). Rather, its dominance is due exclusively to superior independent sporulation as well as its ability, at high frequencies, to decrease the efficiency of GJV2 sporulation. At low frequencies, OC is also superior to GJV2, but its superiority is due exclusively to mixing-specific exploitation of GJV2. Thus the evolutionary transition from OC to PX was a restoration of social independence that resulted in a loss of ability to exploit GJV2. Such exploitation, however, is not necessary for PX to be socially dominant.

One mutation restores social development

The rapidity of the evolutionary transition from OC to PX suggested that a single mutation of large phenotypic effect may have caused the observed adaptive jump in social and developmental complexity[41]. To identify mutations potentially responsible for restoring social independence in PX, the PX genome was sequenced to ~19-fold coverage[42]. Only a single mutation was found that distinguishes the PX genome from that of its immediate OC ancestor (out of 15 total evolutionary mutational differences between PX and GJV1, the parent of GJV2 and direct ancestor of OC)[42]. This PX-specific mutation altered the central position of a seven-base cytosine run to adenosine, and is located 128 bases above the annotated start codon of a predicted GNAT-family acetyltransferase[43], one of more than 30 in the *M. xanthus* genome (W. C. Nierman *et al.*, unpublished work). The roles of this and other GNAT-acetyltransferases in *M. xanthus* physiology and development have not been characterized. The mutation is absent from all 27 OC-derived clones isolated before the D4 crash that fail to sporulate as clonal cultures. In contrast, all 27 clones isolated from after the D4 crash that exhibit the PX phenotype were found to bear this mutation, which strongly suggested a causal relationship between the mutation and the PX phenotype.

Nonetheless, the PX phenotype might have been conferred by reversion of a mutation present in OC back to its ancestral state in GJV1. Such mutations would not have been detected by sequencing the PX genome, which was compared to the ancestral genome template[42]. To directly test the candidate mutation's function, a markerless transfer of the mutation from PX to GVB207.3 (the immediate unmarked parent of strain OC, see Methods) was performed. A counter-selectable marker system was employed[29,44] in which a plasmid bearing the PX mutation and surrounding sequence was integrated into and then excised from the GVB207.3 genome, thus resulting in clones with either the exact PX sequence (GVB207.3/PX+) or the ancestral sequence (GVB207.3/PX−). Three independently isolated GVB207.3/PX+ clones all showed the PX fruiting and sporulation phenotype (Fig. 5). In pure culture assays comparing spore production by PX, the GVB207.3/PX+ clones and GJV1, the PX and GVB207.3/PX+ genotypes were both superior to GJV1 (8- and 12-fold, respectively, $P < 0.01$ in both cases, *t*-test), but were not significantly different from one another ($P = 0.19$). Three GVB207.3/PX− excisants retained the ancestral base at the mutation site and produced no fruiting bodies or spores (Fig. 5).

The PX mutation may confer its phenotype through *cis* regulation of the developmental expression of the acetyltransferase gene immediately below the mutation. During vegetative growth (and hence at the onset of starvation), expression of this gene was fivefold higher in PX than in a kanamycin-resistant sister-variant of strain GJV2 ('GJV10') and threefold higher than in OC (Fig. 6). Expression remained significantly higher in PX than in GJV10 after six (fourfold difference) and twelve (twofold difference) hours of starvation ($P = 0.033$, 0.0072 and 0.0068 for comparisons at 0, 6 and 12 hours, respectively, *t*-tests on $\log_{10}$-transformed data) (Fig. 6). Expression in PX increased slightly over the first six hours of development but decreased thereafter to only 65% of its initial level after 24 hours. In contrast, expression in the developmentally proficient ancestral variant increased steadily over 24 hours, after which it slightly exceeded expression in PX. Interestingly, expression of the acetyltransferase in OC is not defective relative to GJV10, but rather was significantly higher (1.7-fold difference, $P = 0.0035$) at the onset of starvation, after which it increased gradually to levels similar to the other two strains at 24 hours. If the increased acetyltransferase expression in PX is the cause of developmental restoration, it represents a novel transcriptional pathway by which proficient development can be accomplished.

Increased developmental expression of this acetyltransferase may cause developmental restoration in PX by modifying the expression of many other developmentally-regulated genes via an uncharacterized genetic pathway. Preliminary quantitative real-time PCR (polymerase chain reaction) studies of several genes with development-specific expression patterns show that expression of these genes during development changed greatly in the transition from OC to PX, indicating that the transition mutation has many and diverse regulatory effects (data not shown). Moreover, expression patterns of these genes often appear to differ significantly between GJV10 and PX, suggesting that PX development is associated with unique developmental transcription patterns that may be functionally significant.

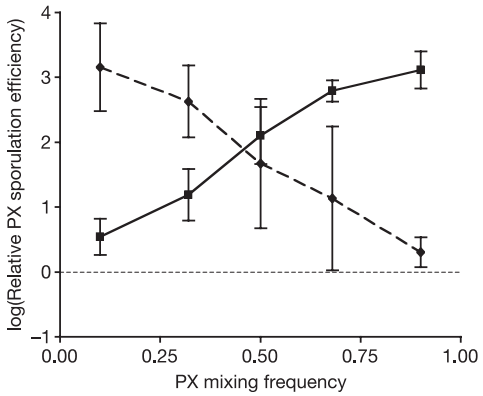

Figure 3 | Log-transformed ratios of PX sporulation efficiency relative to that of GJV2 and OC in direct pairwise competitions at multiple mixing frequencies. The sporulation efficiency of a strain is the frequency of its population at the onset of starvation that survives development as stress-resistant spores. PX sporulates more efficiently than both competitors (GJV2, solid line, and OC, dashed line) at all mixing frequencies (that is, the log-transformed values of PX relative efficiencies are positive in all cases). The horizontal short-dashed line indicates the value (zero) at which two competitors would have equal developmental fitness. Error bars indicate 95% confidence intervals.

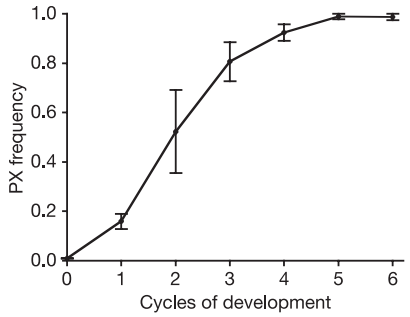

Figure 4 | Frequency of PX in direct competition with GJV2 over six cycles of development. The initial PX frequency was 0.01 and subsequent estimates were made after harvesting spores at the end of each round of development. Error bars indicate 95% confidence intervals.

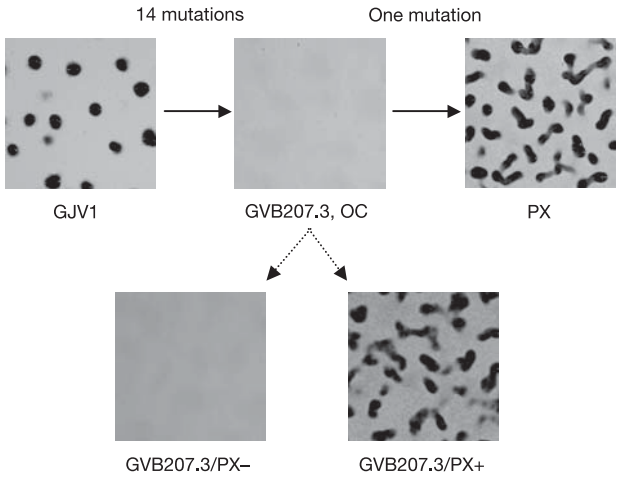

Figure 5 | Fruiting morphology effects of the PX-specific mutation. Strain GVB207.3 accumulated 14 mutational differences from its ancestor GJV1 over 1,000 generations of evolution, whereas strain PX was generated by only a single mutation during ~60 generations of evolution from strain OC (a marked derivative of GVB207.3). Transfer of the PX-specific mutation into the GVB207.3 genomic background fully confers the proficient PX fruiting and sporulation phenotypes (lower right, GVB207.3/PX+), whereas control strains lacking the mutation retain the defective GVB207.3 and OC phenotype (lower left, GVB207.3/PX−).

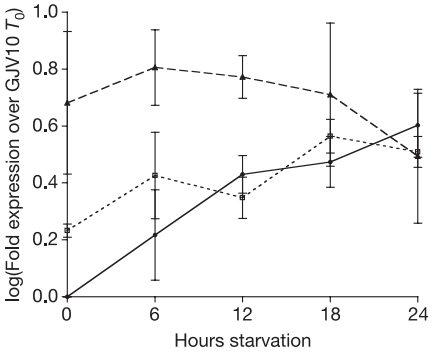

Figure 6 | Developmental expression of the putative acetyltransferase immediately downstream of the PX mutation. Expression levels are log-transformed estimates of fold-differences between GJV10 (a marked variant of GJV1, solid line), OC (dotted line) and PX (dashed line) at various starvation time-points relative to the expression level of GJV10 at the onset of starvation (T_0). Error bars indicate 95% confidence intervals.

The discovery of this regulatory mutation reveals a genetic pathway allowing multicellular development that had not been identified by traditional screens for mutations that suppress developmental defects. This outcome highlights the power of experimental evolution, combined with effective mutation-identification methods, to not only illuminate evolutionary dynamics at both population and molecular levels, but also to generate novel insights into the basic biology of model organisms. It also highlights the variety of pathways by which cooperative behaviours might evolve. Development in PX was not restored by direct reversion of a loss-of-function mutation, but rather by a novel mutational pathway. This evolutionary transition has some similarities to a previously reported restoration of a defective social phenotype (an engineered defect in *M. xanthus* social motility) that was accomplished by compensatory mutation(s) that generated a novel mechanistic basis for an adaptive cooperative trait[29].

The importance of genomic background for expression of the PX phenotype is unclear. Ongoing studies seek to identify which of the 14 mutations that occurred during the 1,000 generations of evolution from GJV1 to GVB207.3 are responsible for developmental incompetence and cheating ability in OC, and whether the PX mutation requires epistatic interaction with any of these mutations to confer the PX phenotype. It will be of further interest to determine whether the PX mutation restores development only in genotypes with the same type of developmental defect as that of OC or is also able to confer developmental proficiency to a broader range of defective genotypes.

The likelihood of evolving out of an obligate relationship should decrease as a function of time spent in the obligate state. Gene functions provided by the sponsoring host or partner should be lost over time in the dependent lineage via selection or drift, making reversion to functional independence increasingly difficult. Before reaching an evolutionary point-of-no-return during obligate dependence, however, some mutations may generate novel independent forms that dominate over competing genotypes. Our results suggest that some extant lineages of free-living organisms may have emerged from temporary evolutionary periods in an obligate state by simple mutational pathways. The relative roles of mutations with small versus large effects on fitness and phenotype in determining evolutionary outcomes have long been investigated[45]. The rapid transition from obligate cheating to autonomous social dominance reported here adds to our understanding of possible tempos and modes of adaptive evolution in social and developmental systems.

METHODS

Strain GJV2 is a spontaneous rifampicin-resistant mutant of GJV1, which is a clone of the standard laboratory strain DK1622 (ref. 46). (GJV2 corresponds to 'R' and 'WT3' in refs 30 and 36, respectively.) Strain GVB207.3 ('S2' in ref. 35) is a 1,000-generation descendant of its ancestor GJV1 (refs 30, 35). Strain OC in this study (also 'S2/pDW79/–' in ref. 35) is a kanamycin-resistant derivative of GVB207.3 generated by chromosomal integration of the plasmid pDW79 (refs 35, 47). The ability of OC to cheat is apparently not caused by pDW79, because a rifampicin-resistant mutant of GVB207.3 (with no plasmid) also exhibits cheating (data not shown).

The original competition in which PX evolved from OC was conducted as described in ref. 36, as was the subsequent competition between PX and GJV1 (Fig. 4) in which PX began as 1% of the population in three independent replicates. Briefly, mixed populations of 5×10^8 cells underwent five days of starvation on buffered agar and were then harvested and heated to kill non-spores. Half of the remaining spore population underwent germination and growth in liquid medium, whereas the other half was sonicated and diluted into selective agar to obtain spore counts of each competitor. This protocol was repeated for multi-cycle competitions. The single-cycle competitions between PX and GJV1 and between PX and a spontaneous rifampicin-resistant mutant of OC (Fig. 3) were performed in three replicate blocks. The presence or absence of the candidate mutation for the transition from OC to PX was examined in 54 OC-derived clones by standard PCR amplification and sequencing of the surrounding region.

Markerless allele exchange. Strain PX was sequenced to ~19-fold average coverage by 454 Life Sciences[42] and reads were assembled against the complete 9,139,763 base pair (bp) genome sequence of strain DK1622 (GenBank accession CP000113). The sole discovered mutation unique to PX is at position 1,258,238 and is 32 bp upstream from a predicted GNAT-family acetyltransferase. A PCR fragment of the PX genome extending 452 bp above and 657 bp below the PX mutation was ligated into the cloning vector pCR-Blunt (Invitrogen), which bears a kanamycin-resistance gene, to generate pNY-PX.1. A *Bam*HI-*Eco*RV restriction fragment of pNY-PX.1 containing the cloned region was ligated into the plasmid pBJ113 (ref. 44) digested with *Bam*HI and *Hinc*II to create pNY-PX.2. Plasmid pBJ113 carries kanamycin resistance and *galK* genes that allow selection for plasmid integration and screening for vector excision, respectively, resulting in allele exchange[44]. Strain GVB207.3 was electroporated with pNY-PX.2 and transformants with the integrated plasmid were selected on kanamycin agar and several transformants were plated onto 1% galactose-CTT agar. With the *galK* gene as a counter-selectable marker, galactose-resistant/kanamycin-sensitive colonies were obtained and screened for the PX mutation replacement in GVB207.3 by PCR and sequencing. Plasmid excision resulted in two markerless excisant types that either returned to the original GVB207.3 sequence (GVB207.3/PX−), or have the exchanged PX mutation (GVB207.3/PX+). The developmental morphology and sporulation levels of these excisants were compared to GJV1, GVB207.3, OC and PX as described above.

Real-time PCR. Developmental gene expression of the acetyltransferase downstream of the PX mutation was compared across PX, OC and GJV10, a derivative

of GJV1 marked with kanamycin resistance by transformation with the same plasmid (pDW79) used to generate strain OC from GVB207.3. Development was initiated as in the population assay, except that 16 100-μl aliquots of resuspended culture per strain were deposited on buffered-agar plates for each time point harvest. Samples were harvested at 6, 12, 18 and 24 h in 1.5 ml TPM liquid[48] and transferred into 6 ml of Bacteria Protect Reagent (BPR, Qiagen). Fruiting bodies were dispersed, incubated at room temperature for 10 min, centrifuged at 4,000 *g* for 10 min (4 °C) and frozen after supernatant removal. For the T_0 (immediately prior to starvation) sample, a centrifuged sample of the original liquid culture used to initiate development was resuspended in BPR and processed in the same manner.

To isolate RNA, frozen pellets were thawed and resuspended in 4 ml of 30 mg ml^{-1} lysozyme (Sigma) prepared in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) for cell lysis. RNA was extracted with a Qiagen Midi Kit. To remove DNA, RNA eluted in 250 ml of RNase free water was supplemented with 25 μl DNase buffer and 12.5 μl of DNase I (Ambion), incubated at 37 °C for one hour, and cleaned using a Qiagen clean-up kit. Quality was checked by reverse transcriptase-PCR using the forward primer CTCACTCACCCATAAAATC ACCC and reverse primer CCTCGAAGGCCTCCTGGA.

Two μg of RNA treated with DNase I was used to synthesize random complementary DNAs using a reverse transcriptase kit (Applied Biosystems). Subsequently, real-time PCR reactions were prepared by adding 23 μl of premix in the wells of a 96-well optical PCR plate (Applied Biosystems) and then adding 2 μl of cDNA along with primer at a final concentration of 100 nM of gene-specific forward and reverse primers and 1x SYBR Green Mastermix (ABI). 25 μl reaction mixes were placed in an Opticon Cycler (MJ Research), incubated at 95 °C for 10 min and subjected to 40 cycles of 15 s at 95 °C and 60 s at 60 °C, followed by one cycle of 15 s at 95 °C, 30 s at 60 °C and 15 s at 95 °C. The experiment was carried out in three independent biological blocks and two temporally independent technical replicates per block.

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1. Pierce, N. E. *et al.* The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* **47,** 733–771 (2002).
2. Keeling, P. J. & Fast, N. M. Microsporidia: biology and evolution of highly reduced intracellular parasites. *Annu. Rev. Microbiol.* **56,** 93–116 (2002).
3. Alexander, J., Satoskar, A. R. & Russell, D. G. Leishmania species: models of intracellular parasitism. *J. Cell Sci.* **112,** 2993–3002 (1999).
4. Williamson, V. M. & Gleason, C. A. Plant-nematode interactions. *Curr. Opin. Plant Biol.* **6,** 327–333 (2003).
5. Pellmyr, O. & Krenn, H. W. Origin of a complex key innovation in an obligate insect-plant mutualism. *Proc. Natl Acad. Sci. USA* **99,** 5498–5502 (2002).
6. Lopez-Vaamonde, C., Rasplus, J. Y., Weiblen, G. D. & Cook, J. M. Molecular phylogenies of fig wasps: partial cocladogenesis of pollinators and parasites. *Mol. Phylogenet. Evol.* **21,** 55–71 (2001).
7. Moran, N. A. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* **108,** 583–586 (2002).
8. Moran, N. A. Tracing the evolution of gene loss in obligate bacterial symbionts. *Curr. Opin. Microbiol.* **6,** 512–518 (2003).
9. Rothstein, S. I. & Robinson, S. K. *Parasitic Birds and their Hosts: Studies in Coevolution* (Oxford Univ. Press, New York, 1998).
10. Lorenzi, M. C., Cervo, R., Zacchi, F., Turillazzi, S. & Bagneres, A. G. Dynamics of chemical mimicry in the social parasite wasp *Polistes semenowi* (Hymenoptera: Vespidae). *Parasitology* **129,** 643–651 (2004).
11. Sumner, S., Aanen, D. K., Delabie, J. & Boomsma, J. J. The evolution of social parasitism in Acromyrmex leaf-cutting ants: a test of Emery's rule. *Insectes Soc.* **51,** 37–42 (2004).
12. Cervo, R., Macinai, V., Dechigi, F. & Turillazzi, S. Fast growth of immature brood in a social parasite wasp: A convergent evolution between avian and insect cuckoos. *Am. Nat.* **164,** 814–820 (2004).
13. Foitzik, S., Fischer, B. & Heinze, J. Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behav. Ecol.* **14,** 80–88 (2003).
14. Pellmyr, O. & Thompson, J. N. Multiple occurrences of mutualism in the yucca moth lineage. *Proc. Natl Acad. Sci. USA* **89,** 2927–2929 (1992).
15. Sorenson, M. D. & Payne, R. B. A single ancient origin of brood parasitism in African finches: implications for host-parasite coevolution. *Evol. Int. J. Org. Evol.* **55,** 2550–2567 (2001).
16. Dale, C., Wang, B., Moran, N. & Ochman, H. Loss of DNA recombinational repair enzymes in the initial stages of genome degeneration. *Mol. Biol. Evol.* **20,** 1188–1194 (2003).
17. Maynard Smith, J. & Szathmáry, E. *The Major Transitions in Evolution* (Freeman Spektrum, Oxford, 1995).
18. Seidler, R. J. & Starr, M. P. Isolation and characterization of host-independent Bdellovibrios. *J. Bacteriol.* **100,** 769–785 (1969).
19. Lutzoni, F., Pagel, M. & Reeb, V. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* **411,** 937–940 (2001).
20. Dawid, W. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol. Rev.* **24,** 403–427 (2000).
21. Rosenberg, E. & Varon, M. in *Myxobacteria: Development and Cell Interactions* (ed. Rosenberg, E.) 109–125 (Springer, New York, 1984).
22. Wu, S. S. & Kaiser, D. Genetic and functional evidence that Type IV pili are required for social gliding motility in *Myxococcus xanthus*. *Mol. Microbiol.* **18,** 547–558 (1995).
23. Shimkets, L. J. Intercellular signaling during fruiting-body development of *Myxococcus xanthus*. *Annu. Rev. Microbiol.* **53,** 525–549 (1999).
24. Wireman, J. W. & Dworkin, M. Developmentally induced autolysis during fruiting body formation by *Myxococcus xanthus*. *J. Bacteriol.* **129,** 796–802 (1977).
25. Fiegna, F. & Velicer, G. J. Exploitative and hierarchical antagonism in a cooperative bacterium. *PLoS Biol.* **3,** 1980–1987 (2005).
26. Velicer, G. J., Kroos, L. & Lenski, R. E. Developmental cheating in the social bacterium *Myxococcus xanthus*. *Nature* **404,** 598–601 (2000).
27. Rainey, P. B. & Rainey, K. Evolution of cooperation and conflict in experimental bacterial populations. *Nature* **425,** 72–74 (2003).
28. Sachs, J. L. & Bull, J. J. Experimental evolution of conflict mediation between genomes. *Proc. Natl Acad. Sci. USA* **102,** 390–395 (2005).
29. Velicer, G. J. & Yu, Y. T. Evolution of novel cooperative swarming in the bacterium *Myxococcus xanthus*. *Nature* **425,** 75–78 (2003).
30. Velicer, G. J., Kroos, L. & Lenski, R. E. Loss of social behaviors by *Myxococcus xanthus* during evolution in an unstructured habitat. *Proc. Natl Acad. Sci. USA* **95,** 12376–12380 (1998).
31. Dugatkin, L. A., Perlin, M., Lucas, J. S. & Atlas, R. Group-beneficial traits, frequency-dependent selection and genotypic diversity: an antibiotic resistance paradigm. *Proc. R. Soc. Lond. B* **272,** 79–83 (2005).
32. Greig, D. & Travisano, M. The Prisoner's Dilemma and polymorphism in yeast SUC genes. *Proc. R. Soc. Lond. B* **271** (Suppl 3), S25–S26 (2004).
33. Strassmann, J. E., Zhu, Y. & Queller, D. C. Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. *Nature* **408,** 965–967 (2000).
34. Velicer, G. J. Social strife in the microbial world. *Trends Microbiol.* **11,** 330–337 (2003).
35. Velicer, G. J., Lenski, R. E. & Kroos, L. Rescue of social motility lost during evolution of *Myxococcus xanthus* in an asocial environment. *J. Bacteriol.* **184,** 2719–2727 (2002).
36. Fiegna, F. & Velicer, G. J. Competitive fates of bacterial social parasites: persistence and self-induced extinction of *Myxococcus xanthus* cheaters. *Proc. R. Soc. Lond. B* **270,** 1527–1534 (2003).
37. Frank, S. A. Perspective: repression of competition and the evolution of cooperation. *Evol. Int. J. Org. Evol.* **57,** 693–705 (2003).
38. Keller, L. *Levels of Selection in Evolution* (Princeton Univ. Press, Princeton, New Jersey, 1999).
39. Travisano, M. & Velicer, G. J. Strategies of microbial cheater control. *Trends Microbiol.* **12,** 72–78 (2004).
40. Foster, K. R., Shaulsky, G., Strassmann, J. E., Queller, D. C. & Thompson, C. R. L. Pleiotropy as a mechanism to stabilize cooperation. *Nature* **431,** 693–696 (2004).
41. Lenski, R. E., Ofria, C., Pennock, R. T. & Adami, C. The evolutionary origin of complex features. *Nature* **423,** 139–144 (2003).
42. Velicer, G. J., *et al.* Comprehensive mutation identification in an evolved bacterial cooperator and its cheating ancestor. *Proc. Natl Acad. Sci. USA* (in the press).
43. Vetting, M. W. *et al.* Structure and functions of the GNAT superfamily of acetyltransferases. *Arch. Biochem. Biophys.* **433,** 212–226 (2005).
44. Rodriguez, A. M. & Spormann, A. M. Genetic and molecular analysis of *cglB*, a gene essential for single-cell gliding in *Myxococcus xanthus*. *J. Bacteriol.* **181,** 4381–4390 (1999).
45. Orr, H. A. The genetic theory of adaptation: A brief history. *Nature Rev. Genet.* **6,** 119–127 (2005).
46. Kaiser, D. Social gliding is correlated with the presence of pili in *Myxococcus xanthus*. *Proc. Natl Acad. Sci. USA* **76,** 5952–5956 (1979).
47. Wall, D., Kolenbrander, P. E. & Kaiser, D. The *Myxococcus xanthus pilQ (sglA)* gene encodes a secretin homolog required for Type IV pilus biogenesis, social motility and development. *J. Bacteriol.* **181,** 24–33 (1999).
48. Bretscher, A. P. & Kaiser, D. Nutrition of *Myxococcus xanthus*, a fruiting myxobacterium. *J. Bacteriol.* **133,** 763–768 (1978).

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