

Reviews of manuscript version 1

Editorial Board Member's Remarks to Author:

This is an interesting paper of potential interest to readers. Yet the manuscript could be improved as indicated by the referees. Besides these specific comments, the likely generality or lack thereof of the result should be made more clear.

Reviewer Comments:

Reviewer #1:

Suitable Quality?: Yes

Sufficient General Interest?: Yes

Conclusions Justified?: Yes

Clearly Written?: Yes

Procedures Described?: Yes

Supplemental Material Warranted?: Yes

Comments:

Schaal et al. test the extent to which the fitness effects of a mutation are influenced by social context and by genomic background in *Myxococcus xanthus*. Specifically, they look at a mutation in a gene that produces signals for fruiting body formation, *csgA*. Disruption of this gene allows a lab strain to cheat on a close relative by decreasing the cheat's investment in the fruiting body and increasing its representation in the spore forming cells. First, the authors demonstrate that this mutant cannot cheat on wild isolates. They then show that experimental evolution of the lab cooperator can decrease the ability of the mutant to cheat. Finally, the authors look at the impact of moving the mutation that causes cheating in the lab strain into wild isolates. They find that the mutation has different impacts on spore formation in different backgrounds. In the cases that the mutation disrupts spore formation, the mutant can be rescued by its parent strain but rarely by other wild isolates. The authors marshal this data to suggest that cooperation can be maintained by genetic diversification, and that the genetic basis of a conserved developmental phenotype can diverge across lineages.

In the experiments testing the lab cheater against other genotypes, it is unclear to me whether results are specific to scenarios of cooperation. In some sense if you have any genotype A that is more fit than a genotype B, is it necessarily surprising that A might be less fit than genotypes C to F? Similarly, if you allow genotype B to evolve wouldn't you expect that it will often become more fit than A? It seems like it would help to disentangle social interactions versus other aspects of the genotypic differences by knowing the fitness of GJV1 (the lab cooperator) against the wild isolates and the evolved isolates.

It would also be helpful to see more discussion of the generality of these findings. It seems likely that the extent to which divergence protects against cheaters will be influenced by the nature of cooperation. For example, secretion of public goods may be less protected by divergence than developmental phenotypes. It would also be good to see some more discussion of how the current results relate to previous work on the rapid evolution of cooperative signals (e.g. PMID 16572169). Some work on evolution of signals is cited but there is little discussion of its relation to the current work.

This is a well-written paper and social evolution and evo-devo are certainly of broad interest. It is perhaps somewhat incremental building on some of the senior authors' previous work showing that kin discrimination can rapidly evolve in this system (PMID 26150498).

Minor comment:

Line 176 - The incorrect figure is cited.

Line 178 - It would be interesting to know how the difference in outcome compares to the genetic similarity. Sequences are provided in Figure S3, but it would be helpful to see this data converted to a tree. Similarly, I would be interested to know the similarity of the broader genetic background. Is this

information known?

Reviewer #2:

Suitable Quality?: Yes
Sufficient General Interest?: Yes
Conclusions Justified?: Yes
Clearly Written?: Yes
Procedures Described?: Yes
Supplemental Material Warranted?: Yes

Comments:

In this manuscript by Schaal et al., the authors present data that show cheating effects in *Myxococcus xanthus* are limited to closely related cooperator-cheater pairs and a relatively small genetic divergence can alter the outcome of cooperator-cheater interactions. The authors also find that a cheating mutation in the *csgA* gene confers a cheating interaction in one genetic background but not in others. The authors argue that this inability of this *csgA* mutation to recapitulate cheating in other strain backgrounds is an example of Developmental System Drift in a microbial system, as *csgA* is conserved between strains but seems to have different functions in spore production. In general, I thought the manuscript was well written and clear, although there was some jargon that could be better defined. My only concerns are to what extent other interactions such as competitive antagonisms might be masking cheating interactions, which I've outlined below.

1) The authors state that antagonisms between *M. xanthus* strains are commonly found, and my concern is to what extent is the observed pattern in Figure 1 inhibition by the natural strains vs. the inability of DK5208 to be complemented by the natural strains? I am not familiar when competitive inhibition between strains occurs in *M. xanthus* so this may not be an issue during development. However, if there is some form of inhibition that should be visible when competing the ancestral, cooperative DK1622 strain against the natural strains. Is this strain still able to form spores? However, with that said, I found Figure 2 much more convincing, as it is unlikely that any inhibition between recently diverged strains would have evolved.

Similarly, in Figure 4, to what extent is it the *csgA* mutation is determining the fitness outcomes observed? For example, in panel one when GJV1 without the mutation (or N9, N16, N23) is competed against N2 is it able to produce spores?

This seems to be important as there is presumably a difference between the inability of a cheating strain to be complemented or being actively inhibited via some sort of toxic intermediary.

2) On line 40 in the abstract, it states that genomic divergence determined whether and how much the same mutation reduces a cooperative phenotype. Based on Figure 3, I'm confused by this statement. To what extent is genomic divergence predictive of the effects of the *csgA* mutation?

3) The authors used the term 'cheater-blind' throughout the manuscript without clearly explaining what they mean by this. It would have been helpful if this was defined in the abstract or at the very least in the introduction.

4) On line 200, the manuscript states that the mutants socially exploited their parents. Is it exploitation if there is no fitness consequence on the parental strain?

5) I found the title unclear and confusing. Neither the latency of cheater evolution, nor the genetic requirements were explicitly measured in these experiments. It may very well be that because high cheater loads in wild populations of *M. xanthus* there are strong pre-adaptations or genetic architecture that predisposes diversification of cheatable developmental pathways. This isn't discussed, so again to what extent is this evolution really latent? Also, what are the genetic requirements for a cooperative trait in this system? This isn't explicitly addressed apart from suggesting there are genetic background effects, but that's not necessarily informative about what the genetic requirements are.