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Dear Editors,

We are contacting to inquire about the possibility of submitting our manuscript entitled *"Polyploid cyanobacterial genomes provide a reservoir of mutations allowing rapid evolution of herbicide resistance”* as an Article in *Current Biology.* It provides a significant advancement in our understanding of the emergence of antimicrobial resistance and compelling evidence in support of a functional role for genome polyploidy in cyanobacteria, which is an often talked about but rarely characterised aspect of modern microbial physiology.

There is great interest in understanding and controlling the mechanisms by which bacteria adapt to stressful conditions, such as those induced by xenobiotic chemicals, which are commonly used to enhance or inhibit bacterial physiological processes. It is clear that exposure to negative selection pressures often leads to enrichment of mutant strains encoding adaptive mechanisms circumventing the deleterious effects induced by the xenobiotics. This fact is underscored by the process of biological evolution itself but has serious implications for humans’ ability to control the bacterial world. This is highlighted by the exponentially increasing number of annual deaths related to antimicrobial resistant (AMR) bacteria, which is expected to reach 10 million by 2050 (<https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf>), and by the frequent observations of loss of function in genetically engineered strains that rely on antibiotic selection or inducers that exert selection pressures working against the engineered function (<https://doi.org/10.1111%2F1751-7915.13327>; <https://doi.org/10.1038%2Fs41467-021-23573-3>).

Much of the attention on understanding the mechanisms of microbial adaptation has concentrated on the effect of horizontal gene transfer and the use of monoploid/diploid strains such as *Escherichia coli*. However, axenic cultures of isogenic bacterial populations with no access to environmental reservoirs of AMR genes can rapidly evolve AMR (<https://doi.org/10.1186/1471-2148-8-52>) and, far from the simple textbook description of bacteria as possessing one or two identical copies of their genome, many phylogenetically diverse prokaryotic strains contain multiple non-equivalent copies of their genome (https://doi.org/10.1038/s41564-021-01034-3). Nonetheless, the physiological role and evolutionary consequences of genome ploidy in bacteria remain largely unknown. Relevantly to this, in the article “Ploidy is an important determinant of fluoroquinolone persister survival”, which was published in your journal, Murawski and Brynildsen (2021) demonstrated that diploid *E. coli* subpopulations contained up to 16 times more “persister” cells compared to the monoploid subpopulation upon treatment with fluoroquinolones (<https://doi.org/10.1016/j.cub.2021.02.040>).

In our manuscript, we report that clonal populations of the model freshwater cyanobacterium strain *Synechocystis sp.* PCC 6803, which is highly polyploid, could rapidly evolve resistance to methyl viologen (MV), which is commonly used as a herbicide (paraquat) in agriculture and as an artificial electron shuttle to improve performance in bioelectrochemical applications. We show that MV exerted bactericidal effects through production of reactive oxygen species and performed adaptive laboratory evolution experiments to isolate MV resistant strains. Starting from two phenotypically distinct parent *Synechocystis* substrains, we isolated 8 MV-adapted strains, showing up to 30 times more tolerance to MV than the parent strains. We show that the resistance was long-lasting, even after long-term removal of selection pressure and thus performed whole genome sequencing on all strains to identify the genetic mutations that were likely to be responsible for the observed adapted phenotypes. Interestingly, we observed only a few non-synonymous mutations, all in membrane transporters, and recapitulated the same mutations reported by a previous study performed in a different laboratory. Some of these mutations were also observed in multiple independently adapted strains. Surprisingly, these convergent mutations were also present at low frequencies in the parent genomes in a substrain-dependent manner. This indicates that wild type genomes encode a reservoir of mutations that can become rapidly enriched upon exposure to methyl viologen. These findings go against the current understanding of microbial adaptation, which is thought to mostly rely on the emergence of spontaneous mutations or horizontal gene transfer from a an “environmental resistome” but suggest instead that, in the case of cyanobacteria adapting to methyl viologen, is driven by allelic variation that is segregating within a population (i.e. standing genetic variation, <https://doi.org/10.1038/nrg1523>).

Our work will therefore be of great interest to a wide readership, including those working on microbiology, antimicrobial resistance, evolutionary biology, algal blooms, plant physiology, bioelectrochemistry etc… We therefore believe it is particularly appropriate for the wide readership of *Current Biology.*

We confirm that the article is original and not under consideration for publication by another journal. The results presented in this manuscript are new and have not been published by any of the authors in some other journal. The stated authors have written the article, and all authors have approved the manuscript and agreed with submission to *Current Biology*. The authors have no conflict of interest to declare.

Yours sincerely,

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**Polyploid cyanobacterial genomes provide a reservoir of mutations allowing rapid evolution of herbicide resistance**   
  
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# Summary

Microbial adaptation allows the evolution of a population towards a phenotype that best fits the surrounding environment. As of currently understood, adaptive mechanisms in bacteria, which are generally assumed to be haploid, mostly rely on the emergence of spontaneous mutations or lateral gene transfer from a reservoir of pre-existing variants within heterogeneous subpopulations or the surrounding environment, which become established in the population upon exposure to selective pressures. However, many slow growing bacteria can adapt to stressful conditions much faster than their apparent rate of spontaneous mutations, as well as when grown as isogenic cultures in controlled laboratory conditions with no access to environmental variants. This raises the question of where the source of this variation is and what evolutionary mechanisms enable their fixation within adapted populations. Understanding this is crucial for human’s ability to control the prokaryotic world, as highlighted by the exponentially increasing number of pathogenic bacteria resistant to antibiotics and the uncontrolled proliferation of anthropogenically fostered microbial communities, such as toxic cyanobacterial blooms, that threaten ecosystems worldwide. Here, we show that the model freshwater cyanobacterium, *Synechocystis sp.* PCC 6803, which is highly polyploid, can adapt within the order of days to the potent bactericidal properties of the herbicide methyl viologen (MV) and that the variants that are responsible for the adapted phenotypes were already present, at low allelic frequencies, in wild type populations. By performing several independent adaptive laboratory evolution experiments starting from two phenotypically distinct parent substrains followed by deep whole genome sequencing, we show that low frequency mutations in membrane transporters in the parent substrains became enriched in the resulting MV-resistant mutants in a parent-dependent manner. In the presence of MV, MV-resistant strains could still perform oxygenic photosynthesis, but they did so less efficiently than wild types when MV was absent, suggesting trade-offs in cellular fitness associated with the evolution of MV resistance and thus the observed mutations to be conditionally beneficial. The mode of resistance was demonstrated to be lower intracellular MV accumulation, as shown by electrochemical experiments. Taken together, these results suggest that genome polyploidy, which is often talked about but rarely characterised in bacteria, providing a reservoir of conditionally beneficial mutations facilitating rapid adaptation to stressful conditions.