Our ability to engineer microbial metabolic pathways is improving rapidly, enabling the efficient and sustainable biosyntheses of both bulk and fine chemicals. However, novel enzymes frequently require significant optimization to function efficiently in a new production host. More precisely, optimization of a novel enzyme or pathway is a complex co-adaptation process, as the pathway adapts to its new host and the host to its new pathway. Unfortunately, our pathway optimization efforts are frequently limited by our inability to account for the complexities involved in genome-scale engineering of microbial metabolism.

Natural systems must overcome similar challenges in pathway refinement during the process of horizontal gene transfer (HGT). While biologists have elucidated the mechanisms by which physical DNA can be transferred between organisms, much less is known about the process of adaptation following HGT. As is often the case in metabolic engineering projects, newly-acquired abilities may be costly for the host until they are carefully integrated into existing metabolic and regulatory networks. Using dichloromethane (DCM) metabolism in *Methylobacterium* strains as a model system, we have explored the physiological and evolutionary consequences of acquiring a challenging new catabolic pathway.

Dichloromethane degradation is certainly a challenging pathway: among other stresses, each molecule of DCM consumed produces two molecules of hydrochloric acid as byproducts. We first transferred the dehalogenase necessary for DCM catabolism into a range of naïve recipients. The resulting transconjugants varied in their ability to exploit their new catabolic potential, and phylogeny did not explain this variation in success. For each of the recipients, we used experimental evolution as a forward genetic screen to identify mutations that improved fitness when grown with DCM as the sole carbon and energy source. Sequencing the evolved isolates allowed us to identify these mutations, the stresses that were previously limiting growth, and the biochemical mechanisms used to alleviate those stresses. We find that chloride export limits fitness across our panel of *Methylobacterium* strains and uncover multiple mechanisms by which the cells can increase their rate of chloride transport. This research has important implications both for the analysis of natural examples of HGT as well as the efforts of metabolic engineers to effectively recreate HGT in the laboratory for biotechnological applications.