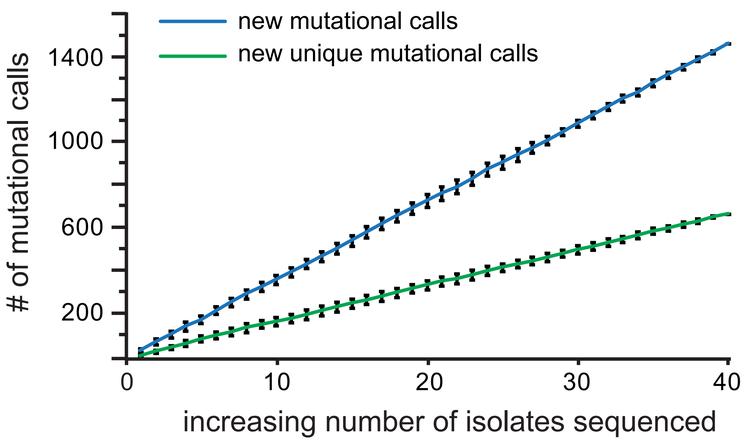
IDENTIFYING DRIVERS AMONG MANY PASSENGERS IN EVOLVING MUTATOR BIOFILMS OF *PSEUDOMONAS AERUGINOSA*

Kenny Flynn* and Vaughn Cooper*
University of New Hampshire, Durham, NH

cooper lab

ABSTRACT

Pseudomonas lung infections of persons with cystic fibrosis (CF) are characterized by **unpredictable pattens of adaptation** following initial diversification. This unpredictability creates highly dynamic *Pseudomonas* infections with rapid turnover of medically important phenotypes. We experimentally evolved three populations from a clone of *Pseudomonas* aeruginosa PA14 for 540 generations under conditions favoring a regular cycle of biofilm formation and dispersal. We found this **long-term regime** was sufficient to recreate this phenotypic and genetic unpredictability. Sequencing of clones and the complete biofilm communities revealed hundreds of segregating mutations and fixed defects in DNA mismatch repair. To dissect this vast genotypic complexity, we used stringent hierarchical clustering of mutations on the basis of affected functional categories to reveal categories of potentially adaptive mutations. This cluster analysis associated with clonal phylogeny and also with changes in phenotypes such as motility and biofilm production. Taken together, numerically **dominant lineages were defined by mutations in major regulators**, which repeatedly gave rise to lineages with specialized traits such as small-colony variants with reduced motility. Regardless of genetic origin, more than 70% of the variation distinguishing these specialists from predominant generalists could be explained by mutations in only a few functional categories implicated in host-association and virulence. This experiment illustrates that adaptive targets may be identified from evolving mutator populations even in the **absence of obvious parallelism**. Moreover, diversity in medically important traits may persist as a byproduct of adaptation in biofilms, which likely contributes to the unpredictability of chronic *Pseudomonas* infections.



biofilms harbor vast genotypic diversity

sequencing 40 isolates does not reach saturation

Despite metagenomic sequencing of replicate biofilm populations with ~400X read depth, sequencing isolates reveals huge numbers of mutations below our 1% detection limit.

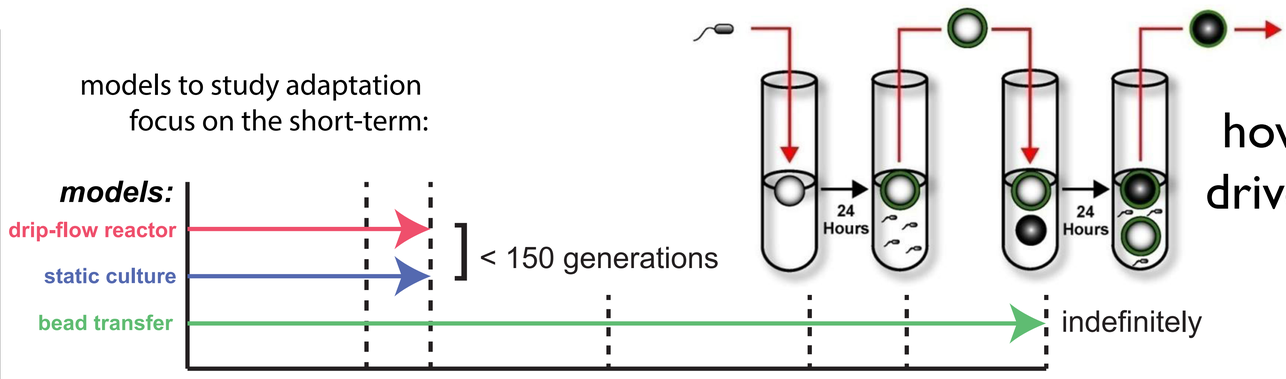
MATERIALS AND METHODS

EXPERIMENTAL EVOLUTION and GENOMIC ANALYSIS. *P. aeruginosa* PA14 was grown on 7mm polystyrene beads suspended in 5 mL of M63 media in a test tube and transferred daily for 90 days in triplicate. The BRESEQ software package (Barrick et al.) was used to align Illumina reads and call polymorphisms for both individual isolates and mixed populations and infer the population genetics of these biofilms from six time points. Functional categories were based on PseudoCAP database annotations. Specific focal mutations examined further through the construction of isogenic mutants created using gateway cloning derived from tools developed by Herbert Schweitzer and Joe Harrison.

ACKNOWLEDGEMENTS

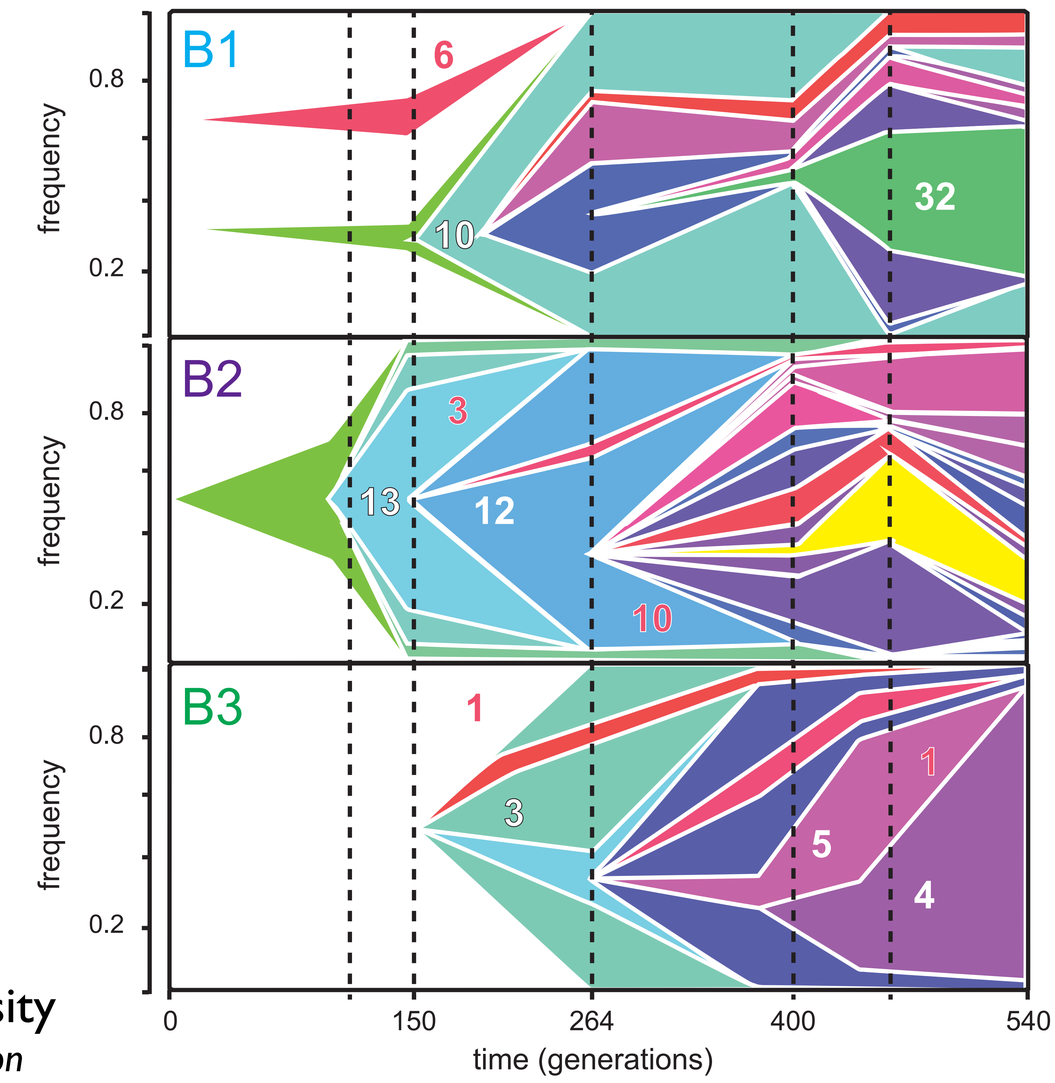
We are grateful to Gabrielle Dowell and Wendy Carlson for technical assistance. We are also grateful to the Lynch lab at Indiana University for help with library construction, the Thomas lab at UNH for providing the raw NGS data as well as the Desai lab at Harvard for assistance with the Fisher-Muller diagrams. We would also like to thank Marcus Dillon and Gabrielle Dowell for helpful conversation and discussion. This work was supported by NSF DEB-0845851 and NHAES.

*Contact Information: kmfynn5@gmail.com, vaughn.cooper@unh.edu

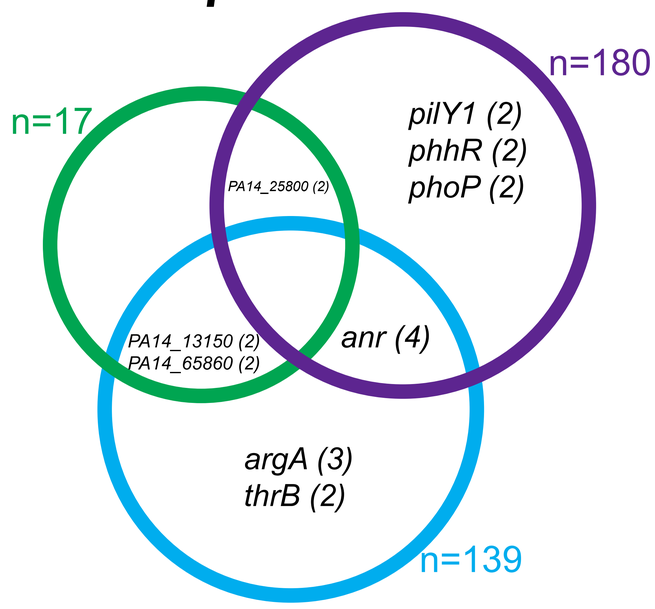


how can we distinguish drivers from passengers?

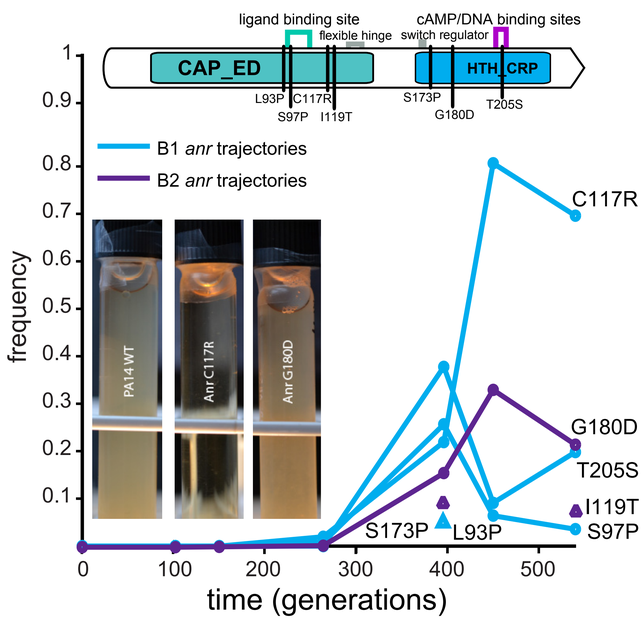
population genetic structure



parallelism



functional convergence reveals common targets but...



mutations associated with ***sweeps*** or ***sways***

shown are mutational numbers associated with a shift in population genetic structure,
white numbers denote 'winners'
red numbers denote 'losers'

anr *mutations do not guarantee success*

parallelism of targets does not identify drivers associated with shifts in population genetic structure