



BIOTIC INTERACTIONS DRIVE CONTINGENT ADAPTATION IN EXPERIMENTAL *PSEUDOMONAS AERUGINOSA* BIOFILMS



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ABSTRACT

Biofilms and associated chronic infections are known to harbor great biodiversity but the consequences of this diversity on community function are not well understood. To understand the forces favoring diversification, 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. A unique set of colony types evolved in each community, and the lack of parallelism was puzzling. We predicted this variation resulted from strongly contingent effects of early variation arising at random, and examined the genomes and ecological strategies of these mutants to evaluate this possibility.

Sequencing of clones and the complete biofilm community revealed that co-existing strains shared a small subset of mutations that fixed in conjunction with a defect in DNA mismatch repair and as many as sixty unique mutations. Small colony variants (SCVs) and auto-aggregative variants evolved in each population, yet isolates of the same colony type through time were genetically distinct, suggesting high standing diversity or recurrent evolution of the same ecological strategies. Similar classes of mutations related to signal transduction, flagella biosynthesis, and production of the biofilm matrix also occurred in each population, albeit at different times. Such variation in the adaptive dynamics is consistent with stochastic forces of community assembly. More evident were the changing targets of selection with prolonged adaptation by the biofilm community. Mutations related to DNA repair and small molecule transport were initially favored, followed by regulatory, metabolism and cell wall / LPS related mutations. This ecological and genetic succession evidently led to strongly co-adapted communities with distinct biofilm phenotypes related to variation in cyclic-di-GMP metabolism. Manipulating cyclic-di-GMP levels within individual mutants in the focal community not only reduced the fitness of the targeted mutant but also drastically altered community composition and destabilized the biofilm in the face of competition.

In summary, these findings suggest that community assembly and adaptation in complex, biofilm environments is largely driven by strong biotic interactions between residents following initially stochastic patterns of colonization and adaptive variation. However, certain targets of adaptation occur in parallel and commonly proceed by altered cyclic-di-GMP metabolism.

MATERIALS AND METHODS

EXPERIMENTAL EVOLUTION. Three replicate ancestral populations of *P. aeruginosa* PA14 were grown on 7mm polystyrene beads suspended in 5 mL of M63 media in a test tube for ninety days or 540 generations (1). Populations were selected for reversible surface attachment by daily transfer of the bead to a new test tube where cells were required to adhere to a new bead in order to persist. Three replicate planktonic lines were also passaged as a control. Morphotypes were isolated and grown in monoculture to confirm heritability of observed phenotypic traits seen on 1% tryptone supplemented with Coomassie blue and Congo red.

COMMUNITY STRUCTURE ASSAYS. Communities were reconstructed by adding representative morphotypes together (based on observed frequencies in the unmanipulated 540 generation B1 community) with substituted complements harboring a vector of interest. For expression of *bifA*, pMQ80-His-*bifA*+ (2), or *sadC* (3), *psadC*, arabinose was added to the media at a final concentration of 0.5%. The consequences of these substitutions were assessed through changes in community structure and fitness relative to the ancestor.

METAGENOMIC DATA ANALYSIS. Population genetics were inferred from a combination of individual re-sequencing of isolates and metagenomic sequencing from three time points. Functional categories were based on the Ausubel PA14 sequencing project. Additional low-frequency mutations detected in the metagenomic dataset or mutations that

REFERENCES

1. Poltak and Cooper. 2010. Ecological succession in long-term experimentally evolved biofilms produces synergistic communities. ISME J. (3):369-78.
2. Kuchma, S. L., K. M. Brothers, J. H. Merritt, N. T. Liberati, F. M. Ausubel, and G. A. O'Toole. 2007. *BifA*, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. J Bacteriol 189:8165-78.
3. Merritt, J. H., K. M. Brothers, S. L. Kuchma, and G. A. O'Toole. 2007. *SadC* reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. J Bacteriol 189:8154-64.

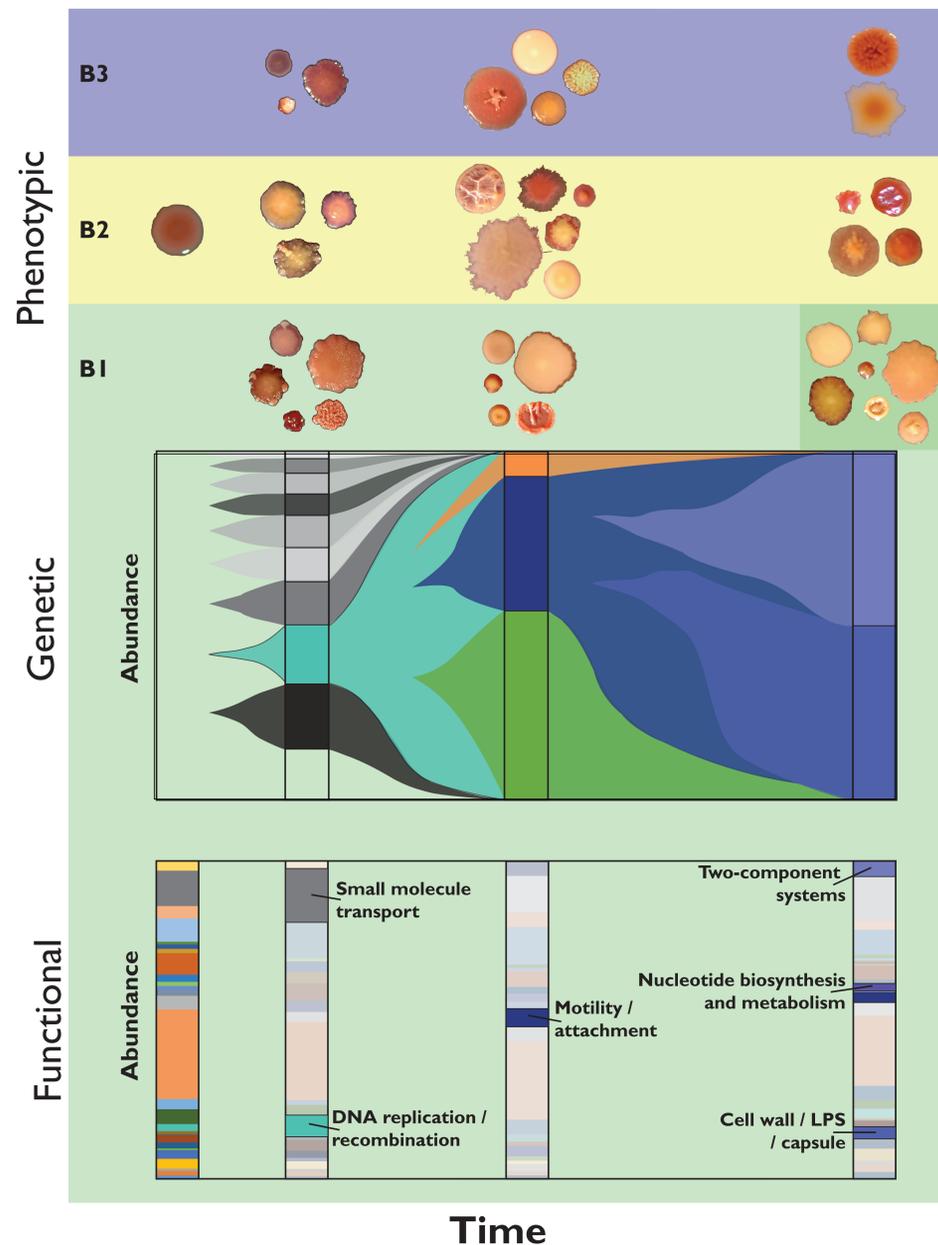


Figure 1. Contingency of ecological succession drives subsequent adaptation and alters the target of selection.

PHENOTYPIC. Community structure fluctuated throughout the experiment with two to seven colony types present with no consistent pattern across replicate biofilm populations (B1, B2, B3). Although similar phenotypes appeared in replicate lines, these phenotypes appeared at different times throughout the evolution, occurred independently, and varied in their ability to persist.

GENETIC. Distinct colors represent competing genetic lineages. Despite the increase in genetic and ecological complexity due to the fixation of a hypermutator allele (teal), a second genetic sweep (blue) occurred as the B1 population adapted suggesting distinct ecological strategies evolve, recurrently.

FUNCTIONAL. Comparing the functional distribution of mutations through time reveals that the targets of adaptation change as the population adapted.

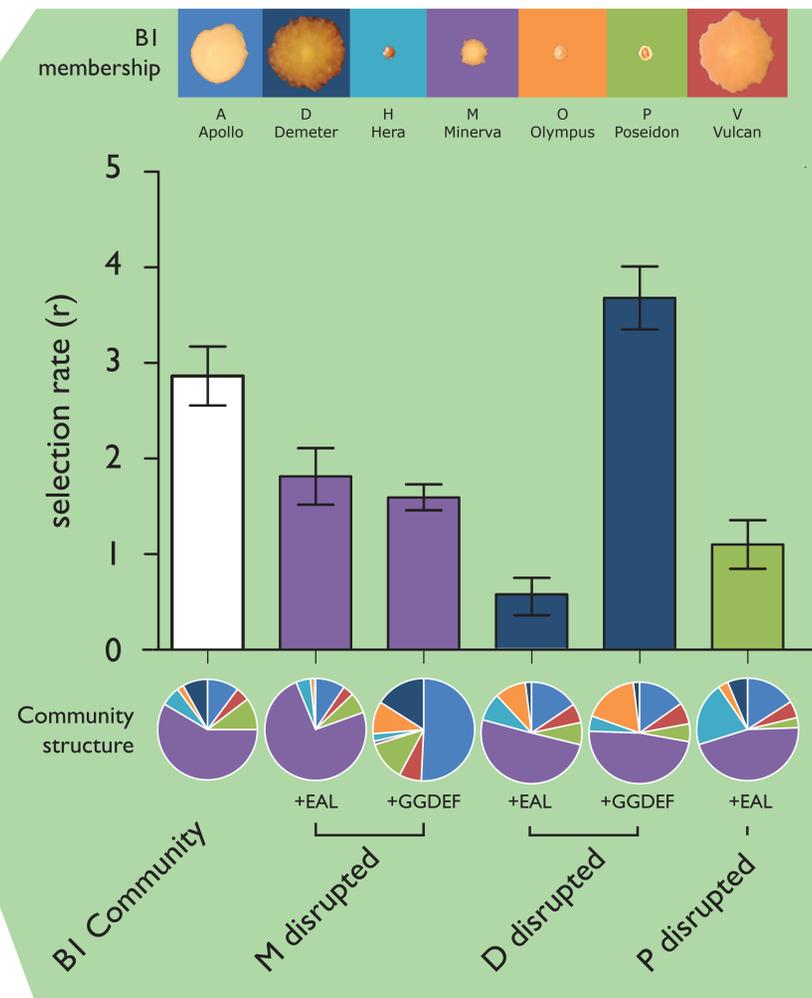


Figure 2. Strong biotic interactions between types allow for rapid changes in community structure

Focusing on the highly diverse B1 population, communities were constructed containing single mutants with artificially reduced (+EAL) or increased (+GGDEF) intercellular concentrations of c-di-GMP. Altering c-di-GMP in a single member produced large effects on the community fitness and structure that were at times more harmful to the community than subtracting that member entirely from the population. Some alterations resulted in rapid succession patterns over only a 24 hour period highlighting the magnitude of the biotic interactions and interdependency in the B1 population.

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