Pleiotropy positively correlates with the direct effect of beneficial mutations

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Abstract

Rare, beneficial mutations increasing fitness provide the foundation by which organisms adapt to their surroundings. However, little is known about the availability of these beneficial mutations, their direct effect on fitness in the selective environment, nor their correlated effects in alternative environments. To assess the spectrum of pleiotropic effects associated with adaptive mutations, we experimentally evolved the cystic fibrosis pathogen *Burkholderia cenocepacia* under conditions selecting for daily biofilm formation and dispersal, collected mutants, and used whole genome re-sequencing to identify their genetic bases. The fitness effects of each mutant were measured in direct competition with the ancestor in the selective environment, as well as a variety of alternative environments. We found that all mutants contained a mutation within a single operon, and fitness was generally positive, although mutants did experience a trade-off in certain environments. Pleiotropy strongly correlated with the magnitude of direct fitness, indicating that mutations of higher initial benefit in the selective environment also dramatically influence fitness in alternative environments, the negative effects of which may bar their success under fluctuating conditions.

Examining how beneficial mutations influence fitness in alternative environments (pleiotropy) may help to better understand how adaptation influences niche breadth, and the types of mutations that are ultimately favored under different conditions. Generally adaptive mutations would likely increase fitness in many environments, while more environmentally specific mutations may have detrimental effects if conditions change. Previous work has shown that beneficial mutations tended to also be adaptive (1, 2) in alternative environments, but that may not be the case for those isolated from a complex, heterogeneous selective environment.

To identify beneficial mutations and measure their pleiotropic effects, mutants were individually isolated from experimentally evolved populations based upon an altered colony morphology known to be associated with biofilm adaptation (3), and their genomes were sequenced.

Twelve unique genotypes were identified, each containing a mutation in one of three genes associated with the Wsp system, a protein complex homologous to the Che chemotaxis system, that is known to influence biofilm formation through production of the messenger molecule cyclic di-GMP (4, 5).

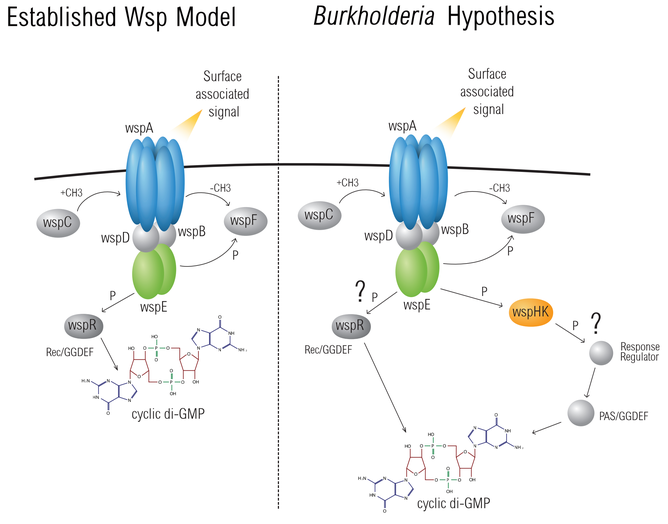


Figure 1: The established model for Wsp associated production of cyclic di-GMP, and proposed function in *Burkholderia*

Methods

Replicate experimentally evolved populations were founded using a 1:1 ratio of Lac marked and unmarked *B. cenocepacia* HI2424. Cultures were grown in 18 x 150mm test tubes containing M9 minimal media supplemented with 3% galactose (3%GMM) and a 7mm polystyrene bead. Alternating beads were passaged every 24 hours; populations were plated on ½ tryptic soy agar with Xgal; mutants were identified by an altered colony morphology known to be associated with biofilm adaptation, isolated, and stored at -80°C.

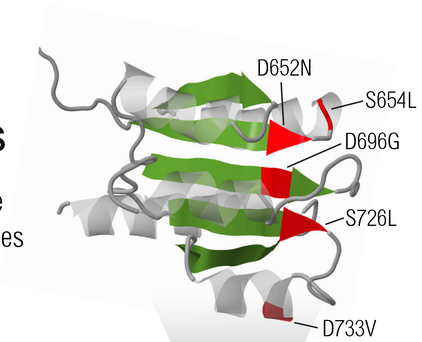
Pair-wise competitions were conducted by conditioning cultures in the competitive envionment, then inoculating each replicate with a 1:1 ratio of mutant and oppositely marked ancestor. After 24 hours, cultures were plated on ½ Tsoy agar plates with Xgal to distinguish mutant and ancestor colonies. Fitness (selection rate constant) was calculated as the difference in mutant and ancestor natural log yield over 24 hours. The absolute value of fitness measured in each environment were averaged for individual mutants to calculate a "pleiotropic index" (PI), and regressed against fitness in the selective environment. All statistics were performed using JMP 10.

Genomic DNA of all 18 mutants and ancestor was prepared using the DNeasy Blood & Tissue Kit, individually barcoded, and sequenced on an Illumina HiSeq 1000. Reads were aligned to a previously sequenced reference genome and mutations called using breseq (Barrick).

The spectrum of pleiotropic effects varies among different genotypes

Pleiotropy varies among genotypes with one of 12 nonsynonymous *wsp* mutations likely affecting phophorelay

Substitutions in the same gene produce mutants with differing colony morphologies and pleiotropic effects



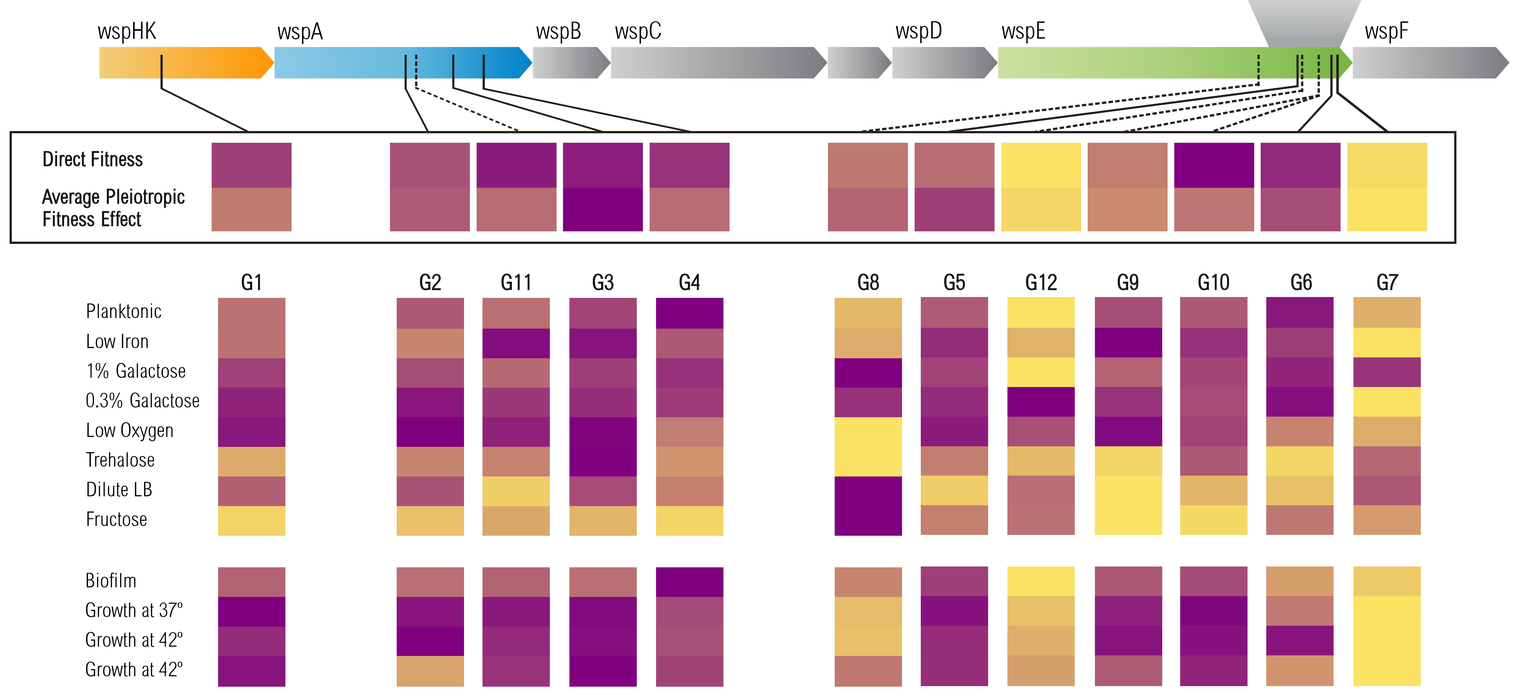


Figure 2: The magnitude of fitness benefit or cost in alternative environments is compared among 12 biofilm-adaptive genotypes.

The magnitude of pleiotropic effect is directly correlated to its initial benefit

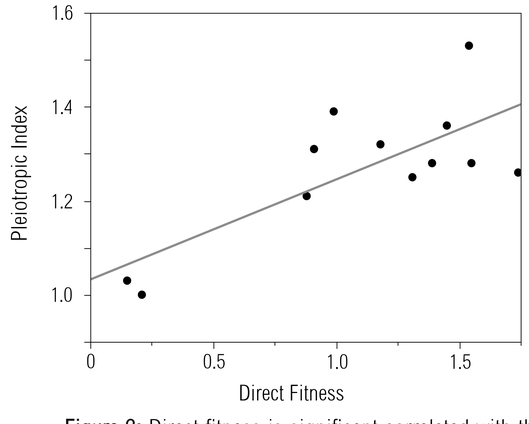


Figure 3: Direct fitness is significant correlated with the averaged magnitude of pleiotropic fitness effect (Pleiotropic Index) ($p = 0.0048$*).

"Generalist" genotypes do not pay as large a fitness cost in disfavored environments, which may influence their success in an adapting population

References

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