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Protein expression tells only half the story

Susanna K Remold

A new mutation in *wspF* allows *Pseudomonas fluorescens* to colonize a previously unoccupied niche, but its proteomic effects reflect only a fitness cost. Between these two observations lies a better understanding of how organisms build and modify networks of protein expression through evolutionary time.

In a study linking 'omics', ecology and macroevolution, Knight *et al.*¹ describe the effects on protein expression of an adaptive mutation responsible for invasion of a new niche. This mutation changes the ancestral broth-dwelling genotype, which forms smooth colonies (SM), into a mat-forming genotype living at the air-broth interface (wrinkly spreading (WS) colonies) (Fig. 1). The authors find that rather than altering expression of proteins involved in mat formation, the mutation affects expression of other proteins, most of which are associated with amino acid catabolism (Fig. 1). These changes might cause a decreased capacity to catabolize specific amino acids, detected by the same authors in previous studies², explaining the observed fitness costs of mat formation.

In 1998, it was shown³ that under very simple conditions (a static tube of media), populations founded with a single genotype of the bacterium *Pseudomonas fluorescens* quickly and repeatably diverge to occupy multiple niches. Among those are the broth occupied by the SM phenotype and the air-broth interface occupied by the WS phenotype. This adaptive radiation (a diversification of one type into many types occupying new niches), although less photogenic than that of Darwin's finches in the Galapagos, may yet inspire as many diverse studies. To date, topics from the ecological conditions that favor diversity⁴ to the cause of the change in phenotype⁵ have been addressed using this system.

Expression, fitness and environment

The focal mutant for this study, a WS genotype called large spreading wrinkly spreader

(LSWS), carries the mutation A901C in *wspF*, which encodes a component of the Wsp (WS-producing) chemotaxis-type signal transduction pathway. Although it is known that WS genotypes overproduce a cellulose-like polymer (CLP) that forms the mat, how *wspF* brings about overproduction of CLP is as yet unknown. Using two-dimensional gel electrophoresis, the authors identified the differences in expression across the proteome between LSWS and its broth-colonizing ancestor. Saturated mini-transposon mutagenesis was thought to have identified all of the mat-forming genes^{1,5}, yet none of these was changed by the mutation. However, 52 proteins differed in expression. Many of these lie in catabolic pathways of substrates on which multiple WS mutants have been shown to grow poorly relative to their SM ancestor^{2,5}. The *wspF* locus thus affects two distinct phenotypes, mat formation and catabolic breadth, although only the latter is demonstrated through protein expression.

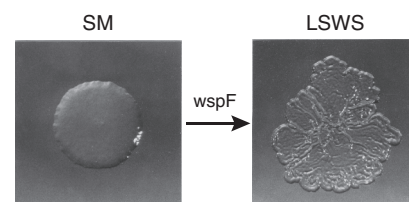
The main result of the study highlights the relevance of ecological context to inference of gene function. If we were to guess the function of the *wspF* allele of LSWS from the authors' proteomic study alone, we would fail to link it to the mat formation module through which it confers an advantage. Instead, we would assign it to one through which it confers a fitness cost. Although one of the roles of the A901C mutation in *wspF* is to change amino acid catabolism, this is no more its 'function' for LSWS than the presence of a large, sturdy beak on a finch is to weigh down its head.

Pleiotropy common in WS mutants

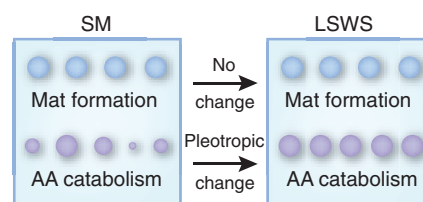
A number of puzzling results of this study may eventually help explain the metabolic and evolu-

tionary links among *wspF*, CLP overproduction and catabolic breadth. Notably, although WS

a Mutation



b Proteome



c Niche

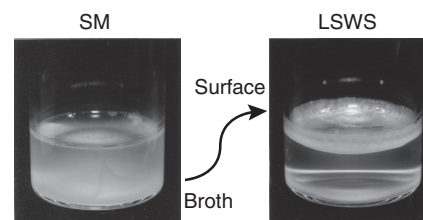


Figure 1 Relationship among mutation, proteome and environment. An adaptive mutation in *Pseudomonas fluorescens* (*wspF* A9016) changes the phenotype (a), influences protein expression (b) and allows colonization of a new niche (c). Although the pleiotropic mutation causes a benefit (mat formation) in the new environment, the proteome tells the story of the mutation's cost. Images in a and c were supplied by P. Rainey and are reprinted from reference 3 with permission.

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genotypes show reduced growth on a number of substrates², culturing LSWS in rich medium increases expression of the relevant enzymes. Not only do most of the pleiotropically affected proteins increase in expression in the LSWS strain relative to the ancestor, but their expression levels are more tightly correlated with one another.

It is possible that WS mutations other than that found in LSWS would cause fewer pleiotropic changes. The authors addressed this by investigating the effects of six additional WS mutations, three within *wspF* and three elsewhere in the genome, on the 52 proteins differing between LSWS and the SM ancestor. They found substantial changes in expression in many, showing that pleiotropic effects occur regularly, regardless of the identity of the mutation. However, network analyses of the protein spots revealed not only strong overall correlations among their effects but also frequent 'outlier' effects. This suggests that, regardless of its particular causal mutation, a new WS genotype

can be expected to show a suite of pleiotropic metabolic changes, although the presence of any particular change will be subject to random chance. Because the authors did not assay known mat formation proteins in these different WS mutants, it is unclear whether failure to cause changes in their expression is characteristic of WS genotypes.

Pleiotropy and evolution

When WS mutants with catabolic deficiencies are allowed to evolve, as shown in previous studies, the emergence of compensatory mutations reduces the cost of the initial mutation in terms of substrate use, without negatively affecting mat formation². By interacting epistatically with both the original WS mutation and downstream loci, such compensatory mutations may break the restriction of the substrate-use phenotype by the allele present at the locus causing mat formation.

What would the proteomic effects of the *wspF* mutation in LSWS be in these compensated genetic backgrounds? Would it show fewer pleiotropic proteomic changes than it does in genetic backgrounds closer to the ancestral SM genotype? Would its expression effects tell us more about how *wspF* affects mat formation or uncover as-yet-unpredicted connections among functional modules? As shown elegantly in the current study, combining evolutionary studies featuring ecologically important mutations with the powerful analysis of networks of interactions common in 'omics' studies brings new insight into the evolution, modification and dissolution of pleiotropically linked traits.

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