Biofilm diversity as a test of the insurance hypothesis

In a recent report, Boles et al. (2004) compared the effect of an environmental stress on high-diversity (wild-type) and low-diversity (recA mutant) biofilm communities of the bacterium *Pseudomonas aeruginosa*. They found increased resistance to an environmental perturbation in the diversified community, which they interpreted as support for the ‘insurance hypothesis’ – an ecological model that predicts that a more diverse community will be better able to resist an external stress. The report is important in that it represents one of very few attempts to examine biofilm communities in the light of ecological theory. Nevertheless we question the authors’ conclusion for two reasons, one general and one specific. We also question the authors’ interpretation that diversification in a biofilm is somehow a programmed response to that lifestyle.

In the ecological literature insurance effects are defined as ‘... any long-term effects of biodiversity that contribute to maintain or enhance ecosystem function in the face of environmental fluctuation’ (Yachi & Loreau, 1999). The mechanism underlying these effects is functional redundancy between community members: a more diverse community is more likely to have members that respond differently to an environmental stress. If there is some amount of redundancy between these members then overall ecosystem functioning can be maintained even if some members fail. This intuitive idea has been around for many years, but was first formally stated in 1999 by Yachi & Loreau. They modelled communities of differing levels of diversity and examined how this diversity affected community productivity in the face of varying degrees of fluctuating environmental stress. They found that increasing community diversity could increase average community function; however, this outcome depended on their being an asynchronous response of the members of the community such that members responded differently to different environmental stresses. For example, member A is more successful than member B in environment 1, but member B is more successful than member A in environment 2 (i.e. a trade-off in response between environments). A key finding of their study was that this trade-off is a necessary aspect of the insurance hypothesis. If member A was more successful in both environments 1 and 2, there would be no difference in community productivity between a monoculture of A and a mixture of A and B.

To test the insurance hypothesis Boles et al. (2004) subjected high- and low-diversity biofilm communities to an oxidative stress. The high-diversity community consisted of three morphological types, ‘wrinkly’, ‘mini’ and the ancestral ‘typical’, whereas the low-diversity community had only the ‘typical’ type. They found that wrinkly members of the high-diversity community were able to withstand this stress while the low-diversity community was killed. This result was interpreted as support for the insurance hypothesis. In our view this observation represents only part of what is required to claim evidence of an insurance effect of diversity. What also needs to be demonstrated is a trade-off over different environments such that some members are better in some environments while others are better in others. Without this demonstration the reported observations do not distinguish between two possibilities: (i) that trade-offs in abilities over environments confer general insurance effects; and (ii) that the wrinkly morphotype is simply a superior competitor destined to take over the community regardless of environment. Only the first possibility is consistent with the insurance hypothesis in providing a mechanism by which diversity can contribute to long-term ecosystem functioning over and above that affected by the best single community member. Designing experiments to unambiguously distinguish between these possibilities is crucial if the power of microbial systems is to be successfully applied to studies aiming to uncover the mechanisms underlying community stability.

Our more specific concern with the experiment of Boles et al. (2004) involves the choice of the environmental stress (peroxide) applied to the high- and low-diversity communities. The stress had already been shown to differentially affect the (pre-diversified) ancestral type and the derived wrinkly type. Thus the finding that the diversified community was more resistant to this stress is not surprising – the stress was chosen on the basis of differentially affecting high and low diversity communities. An unbiased test of the relationship between community diversity and resistance should determine

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Charles Dorman, Editor-in-Chief
the effect of a range of stresses chosen without prior knowledge of their effects on the members of the diversified community.

Finally, we wish to highlight the authors’ discussion of how (and why) diversity originates in biofilms. They consider two explanations for their observation that diversity arises in biofilm but not planktonic communities: that variation is created equally in both communities, but that selection for variant forms is much stronger in biofilms and that there exists some specific ‘...programmed response to the biofilm state...’ (Boles et al., 2004, p. 16635) that generates the observed diversity. Boles et al. (2004) favour the second of these possibilities for two reasons. (i) Communities derived from recA mutants are less diverse than those derived from the wild-type genotype, even though RecA is not thought to be involved in generating growth-dependent mutations. (ii) Auxotrophic mutants reproducibly arise within the biofilm communities in the absence of any obvious selective advantage. Both results can be explained without the need to invoke the involvement of a specific genetic programme. First, recA mutants typically display growth defects (Capaldo et al., 1974; Sciochetti et al., 2001), therefore the low diversity in communities derived from the recA mutant may have little to do with impairment of a biofilm-specific diversity generating mechanism, but rather, be a consequence of the fact that the recA-derived communities have gone through fewer generations compared to the control populations: selection thus has less opportunity to act. Second, as has been shown elsewhere, bacteria adapting to one environment often lose the ability to grow in alternative environments as a pleiotropic consequence of adaptation (Cooper & Lenski, 2000; Cooper et al., 2001; Maclean et al., 2004). In this case, auxotrophy arises as a ‘side-effect’ of adaptation without having to be selected directly. For example, in an experiment in which 12 independent populations of Escherichia coli were evolved in a minimal glucose medium, all 12 populations lost the ability to grow on ribose. This loss was subsequently shown to confer a fitness benefit of 1.5% during growth in glucose. A test of this ‘auxotrophy as a side-effect of adaptation’ hypothesis would be to compare the nature of auxotrophic mutations across independent experiments; parallel loss of function would strongly support there being an underlying adaptive cause (Harvey & Pagel 1991). In the absence of any direct evidence for a programmed response the most parsimonious explanation of biofilm specific diversity is one coupling random variation and strong selection.

The diversity and interactions that can arise in biofilm communities represent unique opportunities for testing ecological and evolutionary theories on a ‘real-time’ timescale. Boles et al. (2004) present interesting observations to this end, but don’t extend these observations to rigorously examine competing explanations. It is precisely these extensions that need to be made if biofilm biology is to incorporate the ecological and evolutionary dimension that is currently lacking.

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