HNSCC local invasion. Tumours like melanomas, which are highly metastatic [20], may use both elongated mesenchymal and rounded amoeboid-like contractile invasion strategies in order to disseminate more efficiently. This plasticity could allow the tumour cell to cope with different environments using a larger repertoire of invasive strategies. Following this line of argument, melanoma patients should be treated with a combination of drugs that inhibit both rounded amoeboid and elongated mesenchymal types of movement [5,7,8,12]. Other tumour types, such as glioblastomas and fibrosarcomas, have been reported to show similar plasticity [11]; therefore, blocking both strategies would also be necessary in order to stop their invasion and/or metastasis [11]. The challenge for the next few years will be to validate tumour invasion signatures as prognosis markers and to find good therapeutic targets within such signatures.

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Population Genomics: How Bacterial Species Form and Why They Don't Exist

Two processes suggested to drive bacterial speciation — periodic selection and recombination — are generally thought to be mutually opposed. Recent work shows that data taken as evidence supporting the former may be explained by the latter, raising further problems for the idea of bacterial 'species'.

W. Ford Doolittle

The concept of species is famously difficult, especially for bacteria. Mayr's 'Biological Species Concept' — that species are interbreeding groups separated from other such groups by reproductive barriers — would not apply to bacteria at all if, as once believed, they are always asexual, never recombining genetically. Obviously, though not trivially, there would be no 'interbreeding'. But some authors maintain that even asexual, non-recombining clones can mimic 'biological species' in important ways. Specifically, ecologically differentiated clonal organisms can maintain relatively constant within-population genomic and phenomic similarity (*cohesion*) over time, while exhibiting increasing between-population *divergence.* An important driver in this *ecotype model* [1] is periodic selection, the operation of which seemed to be favored by earlier data from several groups, including the MIT labs of Martin Polz and Eric Alm. But now these workers offer a serious challenge to the model [2].

Periodic selection, first understood through the chemostat experiments of Kim Atwood [3], is what happens when, in a finite population of non-recombining organisms in a stable niche, a mutant arises that is better able to use the niche's resources. Through selection, all organisms in the population will eventually be the direct descendants of this favored mutant ancestor. And because there is no recombination, their genomes will bear at all loci only direct lineal descendants of those specific alleles the lucky mutant happened to have in *its* genome at the time.

Populations will thus be 'purged' of all allelic diversity accumulated before

the mutation occurred, and if phylogenetic trees are made based on whatever changes have accumulated in genes after that, those trees will all have the same topology. New diversity-purging 'selective sweeps' of this sort will ensue each time a fitter-type mutation appears, so even at marker genes not under selection there will be little persistent sequence diversity. The population stays genetically cohesive within itself, while diverging from other ecologically differentiated populations, experiencing their own niche-driven selective sweeps.

A real-world expectation is that phylogenetic patterns of non-selected marker genes sequenced from an environmental sample (most often phylogenetic tags such as 16S rRNA or hsp 60 genes), will exhibit 'microdiversity' — clusters of many identical or very nearly identical sequences, separated from each other by deep gaps. Clusters should represent 'ecotypes', with whatever internal diversity their genes do show reflecting mutations arising since the last genome-wide sweep. And we'd expect to see some ecological specialization between clusters, if we knew where to look.

In a 2004 survey of 16S rRNA diversity among *Vibrio* species in Massachusetts coastal water samples, Polz's team observed just such microdiverse clusters [4], and four years later Hunt *et al.* [5] began to characterize clusters ecologically, based on the sizes of particles (if any) on which they could be found after filtration (particle size is important to vibrios, many of which grow on or in the bodies of tiny marine invertebrates). They indeed found "numerous ecologically distinct populations at different levels of phylogenetic distinction" within one named species, *V. splendidus*. By the ecotype model, the genomes of different individuals in each hsp60-defined ecologically distinct cluster should show pretty much the same low degree of within-cluster divergence — and pretty much the same (although a higher) degree of between-cluster diversity — in all regions of their genomes, including those bearing the alleles under ecological selection. And all regions should produce the same phylogenetic tree (statistical noise aside).

But now, comparing 13 "L" *V. cyclotrophicus* isolates from large particle habitats and seven "S" isolates from small particle habitats using complete genome sequences, Shapiro *et al.* [2] have found this very much not to be the case. Of more than 29,000 single-nucleotide polymorphisms — SNPs, alternative base assignments among the 'core' genes shared by all 20 genomes — only 725 are 'ecoSNPs' (one nucleotide variant being found in all L strains and an alternative in all S strains). With the ecotype model (or by chance), these should be randomly distributed around the genomes. In fact they are heavily concentrated, 80% found in three small patches and most of the rest in only 11 others, collectively comprising only a few percent of these genomes. Genes in these patches mostly make ecological sense (being involved in biofilm formation, virulence and host colonization, for instance). Contrary to the ecotype model, only those alleles that are responsible for ecological differentiation appear to have been fixed in the two habitat-defined populations.

Diversity at these habitat-specific sites within S or L strains is generally lower than in the rest of their genomes, indicating that the selected regions have spread recently, possibly after their introduction by recombination from donors outside either group. One way to think about this would be that arrival of these new ecologically-relevant genes indeed initiated whole-genome selective sweeps, yet recombination was so frequent and pervasive that long before the genome with the selected allele achieved fixation or even significant prominence, all regions at any distance from that allele had been replaced, often many times over, by recombination with other genomes. Alternatively, as Shapiro *et al.* [2] prefer (E. Alm, personal communication), the eco-selected genes have been introduced independently into multiple recipients in the population. Either way, diversity was not effectively purged.

There is much additional evidence in this paper [2] for rampant within-habitat recombination. Outside the ecotype-specific regions, different genomic segments have different trees, often with mixed clades of L and S types and no single tree favored by more than 1% of genomic length. Interestingly, very recent recombination events, detected by comparing closely related genome pairs, are preferentially habitat-confined (within S or L clades), while older events cross the habitat boundary. It thus appear that the two ecologically differentiated populations are becoming increasingly isolated genetically, increasingly preferring to exchange DNA with their own type, possibly only because of propinquity. One consequence is that, even at unselected markers, the two populations will increasingly exhibit *cohesive divergence*, all genes tending to produce two distinguishable and possibly 'microdiverse' clusters. Such a result would likely have been taken, before the careful work of Shapiro *et al.* [2], as evidence for periodic selection.

In fact, this all looks very much like Mayrian Biological Species behavior, as these authors point out while cautioning that "No matter how marked the decline in gene flow between ecological populations, they will always remain open to uptake of DNA from other populations, thus remaining fundamentally different from biological species of sexual eukaryotes". Moreover, although Shapiro *et al.* [2] don't say this, we must be cautious about generalizing such a result. Recombination depends crucially on biological processes (inducible transformation systems, within-biofilm communication, plasmid behavior, phage availability and host ranges) and physical factors (particle structures and concentrations, hydrodynamic parameters) that are wildly variable and contingent (see, for instance, [6]). There is no reason to suppose that for other bacterial species, or even for *V. cyclotrophicus* at a different time and place, periodic selection might not dominate.

So while efforts like that of Shapiro *et al.* [2] are enormously useful in illustrating or exemplifying how bacterial 'speciation' might occur, their conclusions are not generalizable. There will be no single final answer to the question: 'how do bacterial populations speciate?' or even 'how do they adaptively diverge?' Moreover, by reminding us that two conceptually opposite population genetic processes can mimic each other in producing cohesively diverging genomic/phenomic clusters that some might call 'species', such studies highlight the ontological vacuity of that vexed word.

The 'species problem' is to find a biological process that underwrites and makes 'natural' some particular pattern or extent of clustering, telling us what in general a species *is* and how to recognize one [7]. But if multiple and indeed potentially opposed processes are driving bacterial population genomic evolution with variable intensities and consequences, then there is no reason to expect that all bacteria will belong to clusters at any specified level of cohesiveness, and no non-arbitrary (process-related) criteria for specifying any such level.

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Epithelial Homeostasis: Elimination by Live Cell Extrusion

To maintain a functional and harmonious epithelial society, the number and quality of cells need to be tightly controlled. Two recent studies reveal a novel cellular process for epithelial homeostasis: crowding-mediated live cell extrusion.

Hiroto Katoh and Yasuyuki Fujita*

In epithelial tissues, each cell is connected via tight cell–cell adhesions to form epithelial sheets. To maintain the barrier function of epithelia, the number and quality of cells need to be properly controlled. One classic mechanism that has been intensively studied is contact inhibition, whereby densely populated cells stop proliferating [1]. In addition, studies from our group and others have revealed that cell extrusion is another homeostatic mechanism to eliminate unnecessary (apoptotic) or harmful (transformed) cells from epithelia. When apoptosis occurs in the epithelium, apoptotic cells are recognized by neighboring cells and squeezed out from the epithelial sheet by actomyosin-mediated contractile forces (Figure 1A,A′) [2–4]. When Ras-, Src- or ErbB2-transformed cells are surrounded by normal cells, the transformed cells are extruded from epithelia (Figure 1B,B′) [5–8]. Now, two papers recently published in *Nature* [9,10] demonstrate that there is another way to eliminate cells from epithelia: crowding-induced live cell extrusion (Figure 1C,C′).

During the formation of the dorsal thorax (notum) in *Drosophila*, dorsal parts of wing disc epithelial sheets approach and fuse at the midline where cells become transiently packed. In the first of the new papers, Marinari *et al.* [9] analyzed the behavior and fate of cells at the crowded regions and found that a number of cells near the midline were basally delaminated. The pattern of the delamination varied and was not symmetrical across the midline. Together with other data, which showed that cell lineage, position or developmental time did not play a deterministic role, Marinari *et al.* [9] concluded that this cell delamination is a stochastic process. When cell growth and crowding were enhanced by upregulation of the phosphatidylinositol 3-kinase (PI(3)K) pathway, cell delamination occurred more frequently. In contrast, when the PI(3)K pathway was suppressed, the rates of midline cell delamination were significantly decreased, suggesting that cell growth and density profoundly influence the occurrence of cell delamination.

Furthermore, Marinari *et al.* [9] analyzed the situation in the crowded notum using computational models, assuming that the topological organization of cells changes over time while tissue area is fixed. These models used an equation comprising three parameters: compressibility, junctional tension and cell contractility. By simulating effects of cell density in epithelia, they showed that cell delamination directly correlated with crowding. In addition, by simulating cell geometries in crowded epithelia, they demonstrated that cellular anisotropy promoted delamination. Significantly, by combining these two factors, crowding and geometry, their model phenocopied the process of live cell delamination that occurred *in vivo*. These data indicate that local tissue mechanics are the key factors that determine the tendency of cells to be delaminated.

Via the computational modeling and *in vivo* analyses, Marinari *et al.* [9] demonstrated that there are two patterns of basal cell delamination. In the first pattern, cells gradually lose apical area without concomitant changes in neighbor relationships, keeping cell shapes isotropic. When apoptosis was suppressed by overexpression of the apoptosis inhibitor DIAP1, this pattern of basal delamination diminished, indicating that this process is dependent on apoptosis. In the second pattern, cells progressively lose cell junctions with their neighbors in a stochastic manner, leading to anisotropic cell shapes. These neighbor exchange events and progressive loss of apical area are followed by the recruitment of a contractile myosin II ring within neighboring cells. This pattern was shown to occur for the delamination of live cells but not of apoptotic cells. Thus, delaminations of apoptotic and live cells seem to be governed by distinct molecular mechanisms.