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Host–Microbe Interactions: Winning the Colonization Lottery

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Stochastic bottlenecks during bacterial colonization of animal hosts lead to reduced genetic diversity in the resulting microbiota and, at low-inoculation doses, can result in hosts that remain uncolonized. Bacterial strains vary in their colonization efficiency and resistance to displacement.

The importance of microbial associations for the growth and development of eukaryotic hosts is no longer in question. However, the details of how bacteria–host associations form and perpetuate are unknown in the vast majority of animal and plant symbiotic systems. The microbial communities associated with animals in their natural settings are often predictable at a coarse level and yet are surprisingly inconstant and seemingly somewhat random at finer focus (e.g. [1–3]). Two recent papers have tackled the question of stochasticity in bacterial colonization of the guts of *Drosophila melanogaster* [4] and *Caenorhabditis elegans* [5]. Both papers find that individual bacterial cells have a vanishingly small probability of successfully establishing themselves in the gut — on the order of 1 successful establishment per 1,000 bacterial cells ingested in *C. elegans* — but that those successful few then go on to establish the community for the entire gut. In one of these papers, which appears in this issue of *Current Biology*, Obadia *et al.* [4] fit this to a 'lottery model', wherein a few lucky winners out of a large number of participants reap large rewards.

The lottery model of colonization means that some individual hosts may remain uncolonized even though they have ingested millions of bacteria. Obadia *et al.* [4] use *D. melanogaster* to experimentally demonstrate that these colonization failures are not because the host fly is refractory to being colonized, but instead because every ingestion yields a finite number of bacterial cells playing the colonization lottery and in some cases none win. A further implication is that a single colonized host will sustain only a subset of the bacterial genetic diversity ingested. Vega and Gore [5] provide evidence in support of this prediction using a neutral competition experiment with labelled *Escherichia coli* bacteria to demonstrate that individual *C. elegans* fed on low doses of a mixed culture become arbitrarily colonized by only one of the two bacterial strains. However, both of these properties manifest only when the inoculation dose is relatively low. At higher bacterial inoculations, most or all hosts become colonized [4] and genetic diversity from the inoculum is retained in most individual hosts [5]. This is because even though each individual bacterium has a low probability of successfully colonizing the host, a sufficiently large inoculum will introduce enough cells to produce multiple simultaneous winners (Figure 1).

The findings of Vega and Gore [5] and Obadia *et al.* [4] are made possible because both studies make use of small, inexpensive and easily manipulated model hosts, where each individual worm or fly represents an independent play of the colonization lottery. Because the experiments are high-throughput, inoculation parameters can be readily varied to test the robustness of the colonization outcomes. Both papers then fit the empirical data to mathematical models that capture the quantitative dynamics in a fairly straightforward conceptual framework. The models posit that each individual bacterial cell that is ingested has a low probability of successfully colonizing the gut, meaning that the expected time between successful colonizations is exponentially distributed with an increasing rate of success at larger initial inoculation sizes. Since successful colonists divide and leave descendants, the physical space they occupy is assumed to be unavailable for subsequent invasion. After adding in parameters for post-ingestion rates of bacterial proliferation and cell death or shedding, the models accurately predict the mosaic colonization resulting from large inocula versus the monomorphic colonizations observed with low-inoculation doses.

Although the lottery model of colonization is explicitly stochastic, this does not imply that all bacteria are equivalent in the game. Obadia *et al.* [4] demonstrate that a *Lactobacillus plantarum* strain isolated from a wild-caught fly (WF) is considerably more effective at colonizing the *Drosophila* gut than are strains of the same species isolated from *D. melanogaster* in culture (CS) or from a human gastrointestinal tract (HS). WF successfully colonizes 100% of *D. melanogaster* hosts even when the inoculation dose is as few as 10 bacterial cells, whereas CS and HS strains successfully colonized only 50–70% of hosts at inoculation doses of millions of cells. These outcomes are stereotypical, with 5 out of 6 bacterial species and strains derived from wild flies colonizing at a success rate higher than 90%, compared with only 4 out of 10 species isolated from *D. melanogaster* in culture [4]. Both the Obadia *et al.* and Vega and Gore studies demonstrate priority effects, such that once a bacterial lineage becomes established in the gut, it is not easily displaced by subsequently ingested bacterial cells. Even in this regard, however, not all bacteria are equivalent. Bacterial strains [4] and species [5] vary both in their capacity to invade a colonized gut and in their ability to resist displacement, with the WF strain of Obadia *et al.* [4] showing particular ability

to supplant previously introduced bacteria and to avoid being displaced itself.

The WF strain of *Lactobacillus plantarum* described in Obadia *et al.* [4] would appear to be highly adapted to colonization and persistence in the *D. melanogaster* gut, exhibiting both exceptional capacity to colonize and strong resistance to displacement compared with the CS strain isolated from *D. melanogaster* in culture. An intriguing alternative explanation, however, is that the CS strain is instead exceptionally well adapted to life in the vial. Obadia *et al.* [4] suggest that the WF strain of *L. plantarum* may have greater capability for adhesion to the gut lining, whereas flies inoculated with CS strain shed live bacterial cells at the highest rate. It is reasonable to infer that adhesion is superfluous for strains that experience continuous oral–faecal transmission in a closed space. The most adaptive strategy for bacteria associated with flies in culture may be to quickly flush through the gut and predominantly grow and divide on the *Drosophila* rearing medium [6]. Consistent with this interpretation, *Acetobacter* isolated from laboratory *Drosophila* consistently lose genes encoding flagellar motility and are enriched in the capacity for uric acid metabolism compared with their wild counterparts (P.D. Newell and A.E. Douglas, personal communication). These adaptations are consistent with life in an environment rich in uric acid excreted by flies and where the bacteria can expect passive carriage from vial to vial in perpetuity. *Lactobacillus* and *Acetobacter* strains have previously been shown to be rapidly lost from flies that are frequently transferred to sterile medium and therefore are not able to re-inoculate themselves [7], demonstrating that growth in the medium is crucial for stable association of bacterial strains that evolve with flies in culture.

The differences among bacterial strains and species in colonization efficiency and resistance to displacement raise an important caveat to the colonization models described by Vega and Gore [5] and Obadia *et al.* [4]. These models are defined under the assumption that a naïve, axenic gut is being colonized with a virgin inoculation of bacteria. While the first introduction of bacteria to a sterile host is a defining moment [8,9], what follows is a lifetime barrage of microbes into an established community. Priority effects, facilitation, microbe–microbe warfare, and impacts of host immunity and physiology are likely to be important factors influencing whether an ingested microbe will successfully colonize a mature gut. These will not negate the underlying stochasticity of colonization, but they may bend the odds. Empirical extension of the base lottery model to include direct and indirect interactions among microbes and between the host and potential colonists will require years of additional experimentation.

Nevertheless, an element of randomness in bacterial colonization of eukaryotic hosts is likely to prove to be a universal rule. In addition, stochastic bottlenecks during colonization are unlikely to be limited to the gut. The same principles are likely to apply to colonization of any part of an animal (e.g. [9]) or plant (e.g. [10]), including during pathogenic infections [11]. Indeed, in a parallel to the experimental structure of Vega and Gore [5], we used the standard pinprick assay for systemic infection of *D. melanogaster* [12] to introduce mixed-genotype bacterial infections at low, medium or high dose (~300, 3,000 or 30,000 cells of *Providencia rettgeri*, respectively). Consistent with the results observed by Obadia *et al.* and Vega and Gore, one of the two *P. rettgeri* genotypes randomly dominated each infected fly at the low-dose and medium-dose infections, although both genotypes became represented within individual flies at higher doses of infection (G.M. Fox and B.P. Lazzaro, unpublished data).

It is important to appreciate that bottlenecks in the colonization of the gut or any other body compartment may have diverse mechanistic bases, for example relating to the physics of how bacteria are introduced to hosts, the structural properties of the host or microbe, immunological interactions and resistance, or elements of microbial ecology. These bottleneck forces are certain to vary among diverse hosts and microbes and, crucially, may differ between laboratory and field settings. As phrased by Vega and Gore [5], this "demographic noise" is likely to be very important in the formation of microbial communities as thousands of bacterial cells compete in a colonization lottery that only few can expect to win.

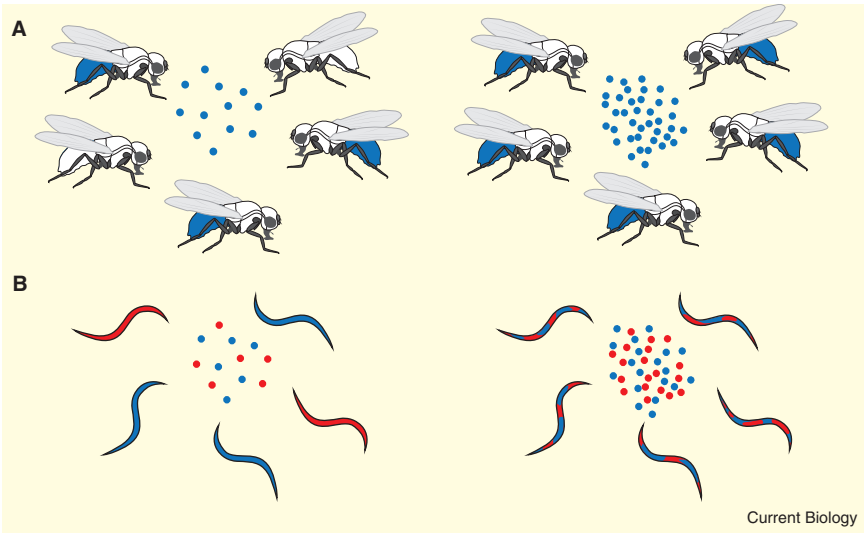

Figure 1. Stochasticity during bacterial colonization of animal hosts.
Because of stochastic bottlenecks, animal hosts that are inoculated with few bacteria become colonized by a single dominant strain or may entirely fail to become colonized. Animal hosts that are inoculated at a high bacterial dose are more likely to become colonized by a mosaic of strains. (A) Obadia *et al.* [4] demonstrated the dose dependency of colonization likelihood by feeding strains of *L. plantarum* to *D. melanogaster*. (B) Vega and Gore [5] fed *E. coli* to *C. elegans* to reveal that mosaic colonization occurs only at high inoculation density.

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Vision: Melanopsin as a *Raumgeber*

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Two new studies show that neural systems receiving inputs from the melanopsin-containing retinal ganglion cells encode spatial information and therefore see the world in more detail than previously thought.

The mammalian eye sees with specialized photoreceptors: the cones, which provide for form, color and motion in daylight; and the rods, which provide for night vision. But light also influences physiology and behaviour beyond sight, for example, entraining our biological clock to the Earth's 24-h rotation and light–dark cycle. This function is mediated by the blue-sensitive photopigment melanopsin, discovered only 20 years ago [1]. Two new studies in mice reported in a recent issue of *Current Biology* reveal that the melanopsin cells of the retina provide more detail than previously thought to the brain areas involved in the perception of images [2] and setting of circadian rhythms [3].

Intrinsically photosensitive retinal ganglion cells (ipRGCs) express the melanopsin photopigment and have large receptive fields, integrating information over space. Because ipRGCs respond to light in a slow and sustained fashion — continuing to fire after a light is turned off — they integrate light over time, creating an ideal system to encode changes in overall light intensity (irradiance). While small in number, the ipRGCs project broadly within the brain (Figure 1A). The suprachiasmatic nucleus (SCN), which serves as the circadian pacemaker in mammals, receives ipRGC input. The ipRGCs also project to the dorsolateral geniculate nucleus (dLGN), the first stop on the route to conscious cortical vision, where the role of melanopsin signals has been less clear. While dLGN neurons respond to melanopsin-only contrast [4], there has been little expectation that the slow, sparse ipRGCs contribute to spatial vision.

In marked contrast to this standard understanding, however, the two new studies demonstrate that the melanopsin system is capable of encoding images. Mouland and colleagues [3] find a population of SCN neurons sensitive to spatial structure, providing a detailed representation of visual space. Further, Allen and colleagues [2] demonstrate that a population of dLGN neurons respond to spatial contrast seen exclusively by melanopsin. Both remarkable findings complement each other in demonstrating a novel role for melanopsin in encoding spatial contrast.

By way of the SCN, light acts as a *zeitgeber*: a signal that aids in synchronizing the internal biological clock to the external illumination. Previous studies [5–7] found that neurons in the SCN preferentially respond to light filling the entire visual field, encoding overall light intensity to track the natural light–dark cycle given by solar illumination. In contrast, Mouland and colleagues [3] find that around 75% of SCN neurons respond to spatial patterns, suggesting the circadian pacemaker has access to the spatial structure of light: a *raumgeber*. By